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CONSTRUCTION OF 2H7 scFv IgG FUSION PROTEINS WITH HINGE MUTATIONS

Additional representative sequences of the present invention are as follows:

HuIgG1 wild type hinge, CH2, CH3

20 tctgatcaggagoccaaattcttgtagcaanaactcacacatgccaccgtgccagacacftgaactctgtggggggagccgtatgtctctct
 cccccaaaacccaaggcacacctcatgatctccggagcccttgaggtacatgcctgtgtgtgtggagctgagccacgaagaccctgag
 gtcaagttcaactgtgtacgtggagcgggtggaggtgcatatgccaagacaagcccgaggaggagcagatcaacaagcacgtatccgt
 tggtcagcgtctctaccgtctctcaccaggagactgtctgaatggcagaagagtacaagtgcaaggctctccaagaagccctccagccccc
 atcgagaanaacaattctccaagaccaaagggcagcccgagagaccacaggtgtgtacacctgtcccccattccgggatgagctgaccaaga
 accagctcagcctgacctgtcgttcaaaaggcttctatcccacatgcctggagagtgaggagcgaatggcagagccggcagaacaa
 25 ctacaagaccagccctccgtctcgtcagatctcgacagctctccctctctctacagcgtacacgtcggtagcagcgtggtagcagacaa
 gaacctgtctctatcgtctgattgcatggagctgtgcacaaacctacacgaggaagcctctccctgtctccgggttaaatgatctaga

HuIgG1 wild type hinge, CH2, CH3

sdqepksodkthtpccpapelggpsvlfppkpkdltmistrtevtcvvdvshdepevkfhwydgvge
hnaaktprreeqynstyrsvsvltvlhqdwlngkeykckvsnkalpapietiskakgqprepqvydppsrdelctknqvsitclvkgy
30 psdiawewesngqpennyktppvldsdgsfflyskitvdksrqwqngvfscsvmhleahnhytqklsislspgk

Llama IgG1 hinge, CH2, CH3

tgatcaagaaccacatggaggatgcacgtgccncagtgccncaatgccngcncngaacnccaggaggc
ccctctctcttctctcccccgaacccaaggacgtcctctccalltttggaggccgagtcacgtgcgttctatgtgcacgtcggaagaaa

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Llama IgG1 hinge, CH2, CH3 (In figure 23 as Llama IgG1)

ephggctcpqcapelpggpsvfvpfpkpdkvlsisgrpvtcvvvdvgkedpevnfnwyidgvevrtantk
pkeeqfnstyrvsvslpiphqdwltgkfcfkcnvnkalpapiertiskaggtrepqvtytlaphreelaktvsvtc/vkgfypadin
ewameqesetgvantpaldndgtvlysrsvgkntwqrgelttgvnmhealnhytksitassgk

15 t g a t c a a g a a c c c a a g n c a c c n a a a c n c n a c c a c a a c c a c c a a c c a a c c a a t c t c a c a a g a a t c a a g t g t c c a a a t g t c a g
c c c c t g a g t c t c t g g g a g g c c c t c a g t c t t a t c t t c c c c c g a a a c c c a a g g a g c g t c t c t c a t t c t g g a g g c c g a g g t c a g t g
c g t g t g g t a g a c t g g g c c a g a a g a c c c g a g g t c a g t t t c a a c t g t a c a t g a t g a t g c g c t g a g g t g g a a c g g c c a a c a c a g g c
c a a a g a g g a a c g t t c a a c a g c a g t a c c g t g t g t c a g c g t c t c g c c a t c a g c a c a g g a c t g g t g a c g g g a a g a a t c a a
g t g c a a g g t c a a c a a a g a g t c t c c g g c c c c a t c g a g a a g a c c a t c c a a g g c c a a a g g c g a a g c c c g g a g c c g a g g t g t a
20 c a c c c t g g c c c a c a c c g g g a a g a g t g g c c a a g g a c a c c g t g a g c g t a a c t a g c t g g t c a a a g g t c t a c c c a c t g a t a t a a n g
t t a g t g c g a g g a a t g g g a c c g g a g t c a g a g g c a g c a c y t a g c a c c a c c a c c c a c c a g c t g g a c a a c a g g g a c a t t c t c t
c t a c a c a g t c t c g g t g g a a a g a c a g t g c a g a g t g a g a a c c t t a c t t g t g t g a t g a c a g g c c t g a c a a c t
a c c c c a a a a t c c a t c a c c a c t c t t c g g t a a a t g t a a t a g a

Dqepktkpqpqpqpntptesckpcapellggpsvfifppkpkdvlslsgrpevtcvvdvgqgedpev
sfnwiyidgaevrtantprkeeqfnstyrvsvlpqhqdwlwtgkfcckcvnnkalpaeiktiskakggtrepqvylaphreelakt
vsvtclkgfyvndinvevwarngapesegattvtpqldndgtvflfyslvgkntwqqgqetfcvnmhealnhhytksitqssgk

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gcacgtaccggtggtcagcgtctctgccatccagcaccaggactggctgacgggaaaggaaatcaagtccaaggtcaacaacaaagt
ctccagccccatcgaggaccatctccaaggccanaaggcagaccggagccgaggtgtacacctggccccacaccgggaa
gagctggccaaaggacacctgtagcgtaacctgctggtcaaggcttctccagctgacntcaacgttgagtgccagaggaaatgggca
gccggagtcagaggccacctacgccaacacgcccacagctggacaacgacgggacctacttctctacagcaaatctccgtggga
5 aagaacacgtggcagcaggggagaagtcttcacctgtgtgtgatgcagggctctacaaatcctccaccagaaatccatccaccg
tcttcgggtaaatgtaatactagaggccc

Llama IgG3 Fc

dqahhsdpsskcpkcpellggptvfifppkakdvisitrkpcvtclwwtvvkdtrssswsvddtevhia
10 etlqpkeeqfnstyrsvlpiqhqlwtlglkfkckvnnkalpapiertiskakgqtreppqvtlaphreelakdvtvstclvkgffpadi
nvewqrngqpesegtyantppqlndngtyflsklsvglntwqqgevtcvvmhealhhnstqksitqssgk

15 *HuIgG1 wild type hinge*

gatcaggagcccaaatctgtgacaaaactcacacatgccaccgtgcccgca

HuIgG1 wild type hinge

dqepksedkhtcpcpa

20

HuIgG1 H2, wild type hinge with leu at second position (results from BglI site)

gntctggagcccaaatctgtgacaaaactcacacatgccaccgtgcccgca

HuIgG1 H2, wild type hinge with leu at second position.

25

dlepksedkhtcpcpa

NT

HuIgG1 wild type CH2

ctgaactcctgggggacgtcagctctctctcccccaccccaaggacacccctcatgatctccgggacccc
30 tgaggtcacatgcggtggtggacgtgagccagaaacctgaggtcaagtcaactggatcgtgagcggcgtggaggtgcataatg
ccaaagacaaggccggaggagcagtacaacagcacgtacctgtgtgacagctcctcaccgctctgcacaggactggctgaatgg
cangggatcaagtccaagtctccacaaaggccctccagcccccagagaaacctatctccaaagccaaa

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HuIgG1 wild type CH2 AA

pellggpsvlfppkpkdtlmistrevtcvvvdshedpevkfiwvydgvvhnaktkpreeqnystyrvvs
vltvllhqdwlngkeykckvsnkalpapietkiskak

5

NT HuIgG1 wild type CH3

gggcagccccgagaaccacaggtgtacacccctgccccatccccgggaggagatgaccaagaaccaggtcagcc
tgacctgccttgctcaaggcttctatccccagcgacatcgccgtggagtgaggagagaalgggcagccggagacaactacaagaccac
gcctcccggtgctgactcgcagcctccttctctatagcaagctcaccgtggacaagagcagggtggcagcagggaactctctca
10 tgcctccgtgagcatgaggctctgcacaaccactacacgcagaagagcctctcctgtccccgggtaaatga

AA HuIgG1 wild type CH3

gqprepqvytlppsreemtknqvsitclvkgyfypsdiavewesngqpennyktppvlidsdgsfflyskltvdsk
15 srwqqgnvfscvmhealhnhytqkslspspk

NT HuIgG1 mutated hinge (C-C-C→S-S-S)

galcaggagcccacaaatctctgacaaaactcacacatccccaccgtccccagca

20

AA HuIgG1 mutated hinge (C-C-C→S-S-S)

dqepkssdkhtspspspa

25

Mutant hinge, but wild type CH2 and CH3—reads from the hinge+Ig tail,
HIgG1MTH WTCH2CH3:

tgatcccccaaatctctgacaaaactcacatctccaccgtcctcagcacctgaactcctgggtggacgcgtcagt
cttcctctcccccaaaaccgaaggacacctcatgatctccggaccctgaggtcacatgcgtggtggacgtgagccacgaaga
ccttgagggtcaagttcaactgctacgtggacggcggtggaggtgcataatgccaagacaaagccgcggggaggagcagtacaacagcacg
30 taccgtgtgtgcagcgtcctaccgtcctgcaccaggactggtgaatgcaaggagfacaaggtcgaaggtctccaaacaaagccctccca
gccccatcgagaanaacatctccaaagccaaaggcagccccggaacacaggtgtacacccctgccccatccccggatgagctga
ccaagaaccaggtgcagctgacctgctggtcaaaaggcttctatccacagcagatcgccgtggagtgaggagcaatgggcagccggga
gaacaactacaagaccagcctcccgctgtggactccgacggctcttctctctacgaagctcaccgtggacaaagagcaggtggca

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gcaggggaacgtctctcatgctcctgcatgcatgaggctctgcacacacacacagcagaagagcctcctctctcctcggglaaatgat
aatctaga

Protein sequence: Mutant hinge, but wild type CH2 and CH3

5 dhpksdkthtspssapellggpsvflfpkpdkdtlmisrtevtcvvvdvshodpcvkfnwyvdgvevhna
lktkpreeqynstyrvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvytlppsdeltknqvsitclvkgyfypsd
iavewesngpennyykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhytqkslsispk

LLG1-5'bg1 35 mer Llama IgG1 5'
10 5'-gtt gtt gat caa gaa cca cat gga gga tgc aag tg-3'

LLG2-5'bg1 32 mer, Llama IgG2-5'
5'-gtt gtt gat caa gaa ccc aag aca cca aaa cc-3'

15 LLG3-5'bg1 33 mer, Llama IgG3-5'
5'-gtt gtt gat caa gog cac cac agc gaa gac cc-3'

LLseqsense 19mer, llama sequencing primer
5'-ctg aga tog agt tca got g-3'

20 LLseqAS 19 mer
5'-oct cct ttg get tig tet c-3'

NT
25 2H7 scFv llama IgG1

aagcttgccgcacatggattttcaagtcgacattttcagcttcctgtaactcagtgcttcagtcataattgccagaggaca
aattgtctctccagcttcacagcaatcctgtctgcatctccaggggagaaaggtcacatgacttcagggccagctcaagtgtaagtacat
gcactggtaccagcagaagccaggtatcctccccaaacccctgatttatgcccatcaacctggctctcggagtccctgctgctgcttcagtg
gcagtggtgtgggacotcttactctctcacaatcagcagagtgaggctgaagatgctgocatttactgccagcagtgaggtttaacc
30 caccacggttcgctgctgggacaaagctggagctgaagatggcggtgctcgggcggtggtggtgctgagggaggtgggagctctca
ggcttatctacagcagctctgggctgagctggtgagggcctggggcctcagtggaagatgtcctgcaaggctctggctacacatttaccagtt
acaatgatgcactgggtaagcagacacctagacagggcctggaatgattggagctattatccaggaatggtgatactctcacaatcag
aagttcaaggcgcaaggcacaatcgtgtagacaatctccagcagacgctcatgacagctcagcagcgtcagctcatctgaagactctgcg

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AA
2H7 scFv llama IgG2

mdfqvqifslflsasvfiargqivlsqspailsaspgkvtmtcrasssvsmhwyqqkpgsspkpwipasn
lasgvparfsqsgstysltisrveadaatyccqwfnsnpptfgagtkleldggsgsgsgsgsgsgsnylqqsgaeivrgpasvk
msckasgytfsynmhwlvktpqrqlgewigaiypngdtsynqkfgkagatlvdkssstaymqllsitedsavvyfcarvvyysn
ywyfdvwtgtttvssddqepktpkpqpqpqpnpntteskcpkcpapelggpsvfifppkpkdvislsgreptcvvvdvqged
pevsfnwyidgaevrantnrpkceeqfistyrvsvlpiqhqdwtlgekfcckvnnkalpapietiskagktrepqvytlaphrecla
kdtvsvtlcvkgfyppdinvewqrngqpesegtyattppqlndgtyflyslkslvgkntwgqgetfcvnmhealnhhytqksitss
ek

2H7 scFv llama IgG3

20 agcgtccgcgcattgatttcaagtgcagallttagcttcgtctaatactggtcttcataaattgcagaggaca
aatgttctctccaggtctccagcaatctcgtctgcatctccaggaggagaagctacaaatgcttcaggcgccagctcaagltgaagttaaat
gcnctgttaccagcagaagcagcagatctctcccanaacccctggatttatgcccaatcaaccttgcttcttgagatccctgtctgcttcagt
gcagltggcttgcggaccttactctctcaaatcagcagagtgggaggctgaagatgtctgccattatctacgcagcagtgaggatttaaac
caccacaggttcgggtctgggacaaagctggagctgaagaatggcgtgtgtccggcggtgtggtatctggagaggtgtggagctctca
25 ggcttatctacagcagctgtgggctgagctgggtgagggcctggggcctcagtgaaagtgtcttgcgaagctcttgggttcaacatttaacagtt
acaatatgcactggtaaaagcacacctagacagggccttgaaatggattgagcttatattctcaggaatgggtatattctctcaaatcag
aagttcaaggcgcaaggccacactgactgtagaacaatctccacgacagcctacatgacgtctcagacgctgacatctgaagcactctgc
gtctatttctgtgcaagagtggtgtactatagtaactcttactgttactctgagttctggggcagcaggaccaggccaacgtctcttctgaltca
agcgaaccacagcgaagacccagctccaaagtgtccaaatggccaggccctgaaactcttggaggggcccaaggtcttcatctctcccc
30 gaaagccaaggagctctctctcatcacccgaaanactggggcactgctgtgtgtgtgagctgggttaagaagcaccctgagatctgagttc
aagctgtgtccgtgtgagcagcaggtgtacacagggctgagaaacacaaaggagacgtgtacacagcgtatccgtgtgtcagc
gtctgtcccatccagcagcagcaggtctgacgggggaaggaattcaaggtcgaaggttcaacaacagctctccagcccccacgagc
gaccatctcagggccaagggcagacccgggaaccacaggtgtacacccctggcccaacccgggaagagctgtggcaaggacacg

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tgagc gtaacctgcctgtcgaaggcttctccagctgacatcaacgttgagtgccagaggaatggcgaccggagtcagagggcacct
acgcc aacacgcgccacacagctggacaacagcgggacacttctctctacagcaaacctccgctgggaagaacacgtgagcagcggg
agaagctctacacctgtgtgtgatgcacgaggctctacacaatactccacccagaataccatcaccagctctcgggttaaatagtaatctag
agggc

5

AA

2H7 scFv llama IgG3

mndfqvqifslisaviargqivlsqspailsaspgekvtmtcrasssvsymhwyyqqkpgsspkpwiypasn
lasgv parfsgsgstysylstrvaedaatyycqwsfnptfagtklclkdggsgsgsgsgsgsgsgaylqqsgaelvrpagsvk
msckasgytfsynmhwwktpqrqlewigalyppngdtsynqkfkgtatlvdksstaymqsltsedsavycarvvyysns
yywfdvwtgtgtvtvsddqahshsdpskcpkcpellggptvfipfkakdvlsitrkpevtclwvwtvktlrsssswsvddt
evhtaetkpkkeeifnstyrvvslpiqhqdwtgkefkckvnnkalpapiertiskakgqtrepyvtilaphrelakdtsvtclvkgf
fpadi nvevqrngqpesegtyantppqldndgtyflyslskvsgkntwqqgevfvcvmhealhnstqksitqsgk

15

2H7+Completely WT IgG tail:

2H7 scFv WTH WTCH2CH3

Nucleotide sequence:

aagcttgcggccatggatttcaagtgacagatttcagcttctgctaatactgcttcacataattgccagaggaca
aatgttctctccagcttcacgaatcctgctgcatctccaggggagaaggtcacatgacttcagggccagctcaagtgtaagttacat
gcactggtaccagcagaagccaggaatcctcccaaaccttgattatgcgccatccaaactggctctcggagtcctcgtcttcagtg
gcagtgggtctgggacctctactctctcaaatcagcagatggagggctgaagatgctgcaactattactgcagcagtggaagtttaacc
caccacgtctggctggggaccgaagctggagctgaaagatggcggtgctcggcgctgtgtagctcgaggaggtgggagctctca
ggcttactctacagcagctcgggctgagctggtagggcctggggcctcagtgaaagatgctcagaggctctgctacacatttaacagt
acaatatgcaactgggtaagcagacacctaagacagggcctgggaatggatggagctattatccaggaatggtgatactctcacaatcag
aagttcaaggccaagggccactgactgtgacaaaatcctcagcacagcctcatgacagctcagcagcctgacatctgaagacitcgcg
glcta tttctgtgcaagagtgctgactatagtaacttactgtacttcgtggtgggcacagggaaccagctcacgcgtctctctgatca
ggagcccaaatctgtgacaaaactcacatgccaccgtggccagcactgaactcctgggggagccgtcagctctcctctccccc
aaacc caaggacacccctatgactctccggaccctgaggtcacatgctgtgtgtggagctgagcccaagacccctgaggtcaagtt
caactggtacgtggagcggcgtggaggtgcataatgccaaagacaaagccgcgggagggagcagtgacaacagcagctaccgtgtgtcagc
gtctcaccgtctcgcacaggagctggctgaatggcgaaggagtacaagtgcaaggctctccaaacaaagccctccagcccccacatcgagaa
aacaatctccaaagccaaaggcgaccccgagaccacagctgtacacccctgcccccacccctggatgagctgaacaaagacagctc
agcc tgaactgcctgtcgaaggcttctatccagcgacatcgccgtgagtgaggagcaatggcgacccggagaaacatacaga

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ccacgcctccgcgtgctgactccgacggctcctctctctacagcaagctcacccgtggacaagagcaggtggcagcaggggaacgtct
tctcatgctccgctgatgatgagcctctgcacaaccaclacacgcagaagcctctccctgtctccgggtaaatgatctaga

2H7+Completely WT IgG tail:

5 2H7 scFv WITH WTCH2CH3

Protein sequence

mdfqvqifslisasviargqivlsqspailsaspgekvtnicrasssvsymhwyqqkpgsspkpwiypsn
lasgvparfsgsgsgtsysltisrveadaatyqcqwsfnpptfgagtdelkdggsgggsgsgsgs5qaylqsgaelvrpgasvk
msckasgytftsynmhwvkqprqglewigaiypngdtsynqkfkfkatltvdksstaymqsltsedsavfyfcarvvyvns
10 ywyfdvwtgttvssdqepkscdkthtppcapellggpsvflfppkpkdtlmisrteptcvvdvshedpevkfnwyvvdg
evhnaktkpreeqynstyrvsvltvlhqdwlngkeyckckvsnkalpapiektiskakgqprepqvyltppsrdeltknqvsltclvk
gfyfysdiawesngqpenyktppvldsdgsfllyskltvdkrswwqgnvfscvmhealhnhytqkslslspgk

NT

15 CD80 transmembrane domain and cytoplasmic tail (+restriction sites)

ggcgatccttgcgaacctgctccatcctgggcattacctaatctcagtaaatggaaattttgtgatgctgcotgacc
tactgctttgccccaaagtgcagagagagaaggaggaatgagagattgagaagggaagtgtacgccctgataaatcgat

AA

20 *CD80 transmembrane domain and cytoplasmic tail*

adpsnllpswaitisvngifvicctycfaprrerrmerlrresvrpv

NT

25 40.2.220 VL (anti-human CD40 scFv #1--VL)

aagcttatggattttcaagtcgacgattttcagcttctcgtactatcagtcgttcacataaagtccagaggagtcgacatt
gttctgactcagtcctcagccacctgtctgactccaggagatagctctcttcttcagcggccagccagagattatgcgactacttac
actggtatcaacaaaatcacatgagctccaaggctctctcaacaaatgcttccattccatcctctggatccctccagtgatgagcagt
ggtacagggtcagatttactctcagtatcaacagtgtagaacctgaagatgttggaaatttactgtcaacatggtccacagcttccgtggac
30 gttcgggtgaggaccaagctggaatcaaacgg

AA

40.2.220 VL (anti-human CD40 scFv #1--VL)

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mdfqvqifslisavimsrgvdivltqspatlsvtpgdrvsiscrasqsisdylhwyqkshesprllikyashsi
sgiprsfsgsgsdftlsinsvepedvgiyycqghghsfpwtfgggtklcikr

NT

5 40.2.220 VH (for anti-human CD40 scFv #1--VH)

cagatccagtttggtgcaatctggacctgagctgaagaagcctggagagacagtcagatctctgc-aagcctctg
ggfatgccttcacaactactgggaatgcagtggtgcaagagatgccaggaaagggttgaagtggattggctggataaacacccactctg
gagtgccaaaatagttagaagacttcaaggacggtttgacctctctttggaaaccttgcacaactgcataattacagataagcaacctcaaa
gatgaaggacacggctacgtattctgtgtgagatccgggaatggtaactatgacctggcctactttgcttactggggccaaagggaactgggt
10 cactgtctctgatca

AA

40.2.220 VH (for anti-human CD40 scFv #1--VH)
qiqlvqsgpelkpgetriskasyaflitgmqvwvqempgklkwigwintplwsalcirrlqgrfafslets
15 antaylqislnkdedatatyfcvrsngnydlafaywgcgtltvts

NT

40.2.220 scFv (anti-human CD40 scFv #1)

20 aagcctatggattttcaagtgacagattttcagcttctgctaalcagtgcttcagtcataatgtccagaggagtcgacatt
gtictgactcagtcctccagccacctgtctgtgactccaggagatagagtcctcttcttcgagggccagccagagtattagcgaactactac
actggtatcaacaaaaacacatgagctccaaggcttctcaataatgtctccattcactctctggatgccctccaggttcagtgacagt
ggatcagggtgcagatttactctcagtatcaaacagtgtagaacctgaagatgttggaattattactgtcaacatggtcacagctttcgtggac
gttcgggtggagggcaccaagctggaaatcaaacgggtggcgggtgctcggcgagggtgggtgggtggcggcggttcagatcca
25 gttgggtgcaatctggacctgagctgaagaagcctggagagacagtcaggaatctctcgaaggctctcggatgctcctcaacactactgga
atgcagtggtggcgaagagatgccaggaaagggttgaagtggattggctggataaacacccactctggagtgccaaaatagtgaanga
cttcaaggacgggtttgccttctcttggaaacctctgccaactgcataattacagataagcaacctcaaaatgaggacacggctactgtatt
tctgtgtgagatccgggaatggtaactatgacctggcctactttgcttactggggccaagggaactgtgactgtctctgatca

AA

30 40.2.220 scFv (anti-human CD40 scFv #1)

mdfqvqifslisavimsrgvdivltqspatlsvtpgdrvsiscrasqsisdylhwyqkshesprllikyashsi
sgiprsfsgsgsdftlsinsvepedvgiyycqghghsfpwtfgggtklkrrggsgggsgggsgggsggiqlvqsgpelkpgetrisk

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ckasgvaftttgmqwvqempgkglkwigwintplwsakicrlqgrfafsletsantaylqisnlkdedytfcvrsngnydlayfa
ywgqgtlvts

NT

5 2e12 VL (with L6 VK leader peptide)

atggatttcaagtcagatttcagcttctgctaatacagtgcttcagtcataatgtccagagagtcgacattgtgctc
acccaatctcagcttcttggctgtgtctctaggtcagagagccacatctcctgcagagccagtgaaagtgatgaatattatgcacaagttt
aatgcagtggtaccacaagaacaggcagagccacccaactctctatctgtgcatocaaacgtagaacttgggtccotgccagggtt
agtggcagtggtgtgggacagacttcagctcagctcaacatccatctgtggaggagatgattgcaatgtattctgtcagcaagaataggaa
10 ggttctctggacgttgggtggaggacacagctggaantcaaacgg

AA

2e12 VL (with L6 VK leader peptide)
mdfqvqifslilisavimsrgvdivltqspaslavslgqratisrasesveyyvtslmqwyqqkpgppkllis
15 aasnvesgvparfsgsgsgtdfslnihpveediamyfcqqrkvvpwtfgggtcklekr

NT

2e12 VH (no leader peptide)
cagggtgcagctgaaggagtcaggacctggcctgggtggcgcctccacagagcctgtccatcacatgcacccgtctca
20 ggggtctcattaacccggtatggtgttaactgggttcgacagcctccaggaagggtctggagtggtcgtgggaatgatatgggtgatggaa
gcacagactataaattcagctctcaaatccagactgagcatcaccaaggacaactccaagagccaagtgtttcttaaaaatgaacagctctgcaa
actgatgacacagccagactactctgtgccagagatggttatagtaacttctactatgtatggactactgggtccaaggaacctcagtcac
ccgtctctca(gatctg)

25 AA

2e12 VH
vqqlkesgpglvapsqslsiictvsgfsltygvnwvrppgkglewlgmiwgdgstdynsalksrslsitkdns
ksqvfikmnsqtdtdaryycardgysnfhyvmdywgqgtsvtvss

30 NT

2e12scFv(+Restriction sites)
aagcttatggattttcaagtcagatttcagcttctgctaatacagtgcttcagtcataatgtccagagagtcgacatt
gtgctcaccacatccagcttcttggctgtgtctctaggtcagagagccacatctcctgcagagccagtgaaagtggtgaatattatgtca

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caagtttaatgcagtggtaccacagaaccaggacagccaccacaaactctctctgctgcatccaacgtagnatctggggctccctgc
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5 tcatlaaccggctatgtgtgtaactgggttcgccagccctcaggaaagggtctggagtggtctgggaatgatgggtgatggaagcaca
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cctct(gatcag)

10 AA
2e12scFv
mdfqvqifsfllisavmsrgvdivltqspaslavslgqratiscrasesveyyvtlsmqwyyqkqpgppkllis
aasnvesgvparsfsgsgtdfslnihpveediamyfcqqsrkvpwtfgggtkleikrgggsgsgsgsgsgsqvqlkespgglva
psqslsitctvsgfsltygvnwvrrppgkglewlgmiwgdgtdynsalksrslitkdnksqvfkmnslqtdtdaryycardgys
15 nfhyyvmdywgqgstvtvss

10A8 is anti-CD152 (CTLA-4)
10A8 VL (with L6 VK leader peptide)
atggatttcaagtcagattttcagcttctgctaactcagtgctcagtcataatgtccagaggagtcgacatccagatg
20 acacagctccatctcactgtctgtcatctctgggaggcaaaagtcaccatcacttgcaaggcnaagccaagcattaagaagtatataggttg
gtaccaacacaagcctggaaagggtccaggctgctcatattacacatctacattacagccaggcatccatcaagggttcagtggaagt
ggctctgggagagattatctcagcatcagaaacctggagcctgaagatattgcaactntattgtcaacagatgataatctccattgacg
ttcggctcggsgacaaagttggaataaaacgg

25 AA
10A8 VL
mdfqvqifsfllisavmsrgvdiqmtqspsslsaslggkvitckasqdikkyigwyqhkpqgprlliiyytst
lqpqipsrfsgsgsgrdylsirmlepediatyyccqqydnpltfsggtkleikr

30 NT
10A8 VH (no leader peptide)
gatgtacagcttcaggagtcaggacctggcctcgtgaaacctctcagtcctgtctctcacctgctctgtcactgagcta
ctccatcaccagtggtttctactggaactggatccgacagttccgggaacaaactggaatggatggccacataagccacgacggttagg

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aataactacaaccatctctcataaactgaatctccactctgtagacacatctaaaccagttttcttgaagttagttctgtgactactgag
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tca

5 AA

10A8 VII

dvqlqesgpglvkpsqslstcsvtgysitgfywnwirqfpgnklewmgshishdgrnnynpslinrisitrdtsk
nqflklssvttedatatyfcarhygssgandmydgqgtsvtvss

10 NT

10A8 scFv

aagcttatggaattttcaagtcagattttcagcttctgctaactcagtgcttcagtcataatgtccagaaggagtcgacatc
cagatgacacagtcctccatccctcactgctcgcacatctctgggagggcaagtcacacactgcaaggcaagccaagacattaaagaagtatat
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20 AA

10A8 scFv

mdfqvqifslisavimsrgvdiqmtspsslsagsgkvititckasqdkkyigwyqhkpgkpgprliiyyst
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30 NT

40.2.220-hmtIgG1-hCD80

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AA

20 40.2.220-hmtIgG1-hCD80

mfdqviqifllisavimsrgvdiqltqspatlsvtpgdrvslsrasqsisdyhlwyqqkshesprllikyashsi
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25 kpreeqnystyrsvslvlhqdvlngkeykckvsnkalpapicktiskakgqprepqvlytppsrclctknqslctlvkgfypsadia
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gfviccltycfaprcerrrrnerlrsrvp

NT

30 2eI2seFv- hmtIgG1-CD80 fusion protein

aagcttatggatttcaagtgacagatttcaagcttctgctaatcagtgcttcagtcataatgtccagagagagtcgacatt
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AA
2e12scFv- hmtIgG1-CD80 fusion protein

20 mdfqvqifsfllsasvimrsngvdivltqspaslasvlgqratiscrasesveyvyslmqwyyqkpgqppklilis
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25 lvkgfypsdiavewesngcnpnyktppvldsgsflylsvtktdksrwqqgnvfscsvmeahlnhytqkslspsgkadpsnll
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15 ttaccttaactcagtaaatggattttgtatgtgctgacctgactgttggcccaagatgcagagagagaaagggaagtggagagatt
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10A8 scFv-hmIgG1-CD80

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25 wesngqpennytktppvldsdgsfflyskltvdksrwqgnvfscvmhealnhhytqslslspgkadpsnllpswaitisvngf
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NT

500A2-hmIgG1-CD80

30 atgtgtatacatctcagctccttgggctttactctcttggafttcagcctocagangtgcatagtgctgactcagctc
cagccactctctctaatctctgggaaagagtcacatgacctgtgaagaccagtcagaataatggcacaattctacagctgctacccaaa
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25 viccltycfaprcrerrnmerlirsvrpv

NT

2H7 scFv MTH(SSS)WTCH2CH3

aagctggccgacctgattttcaagtcagatattcagcttctgctaatcagtgcttcagtcacataatgccagaggaca
aattgtctctccagctccagcaatcctgtctgcatctccagggagaaaggtcacaaatgacttgacggccagctcgaagtgaagtacat
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30

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15 2H7 scFv MTH(SSS)WTH2CH3 protein sequence:

mdfqvqfslisaviiargqivlsqpailsapgekvtmterassvsymhwyqqkpgsspkpwiyapsn
lasgvparfsgsgtsysltisrveacdaatyycqwsfnptfgagtkleldggsgggsgggsggssqaylqsgaelvrpgasvk
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HuIGMHncs1 (oligo for CSS)

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac

25 HuIGMHncs2 (oligo for SCS=ncs2)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tgc cca ccg

HuIGMHncs3 (oligo for SSC=ncs3)

gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tgc cca cca cct g

30

hIgWT3xba (3' oligo for above mutation introduction)

gtt gtt tct aga tca ttt acc cgg aga cag gga gag gct ctt ctg cgt gta g

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Vhsr11: (oligo for Leu to Ser at VH11)

gga ggt ggg agc tct cag gct tat cta cag cag tct ggg gct gag tgg gtg agg cc

huIgG1-3' (3' oligo to amplify IgG1 C regions, 3' end of CH3)

5 gtc tct aga cta tca ttt acc cgg aga cag

huIgA/Gchim5 (oligo for pcr#1)

cca tct ccc tca act cca cct acc cca tct ccc tca tgc gca cct gaa ctc ctg

10 huIgAhg-5' (oligo for pcr#2)

gtt gtt gat cag cca gtt ccc tca act cca cct acc cca tct ccc caa ct

huIgA3'

ggt gtt tct aga tta tca gta gca ggt gcc gtc cac ctc cgc cat gac aac

15

2H7 scFv IgAH IGG WT CH2CH3, 2H7 scFv with IgA hinge and WT CH2 and CH3

20 aagcttgccgccatggatttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataattgccagaggaca
aatgtttctctccagctctccagcaatcctgtctgcatctccaggggagaaaggtcacaatgacttcaggcccaagtcagtgtaagtlacat
gcactgggtaccagcagaagccaggatctctcccaaaccttgatttatgcccatccaacctggcttctggagtcctctgtctgttcagtg
gcagtggtgtctgggaaccttactctctcacaatcagcagagtggaagctgaagatgctgccactttactgcagcagtgagggttttaacc
caccnagctgggtgtctgggaccaagctggagctgaagatggcgggtggctcggcggtgtgtgatctggaggaggtgggagctctca
ggcttatctacagcagcttgaggctgagctgtgagggcctggggcctcagtggaagatgctcgcagaggtctctgtacacattaccagtt
25 acaatatgcactgggtaagcagacacctagacagggcctggaatggatggagctattttatccaggaatgtgtatcttcciaaatcag
aagttcaaggggcagccactgactgtagacaatctctcagcagccctacatgcagctcagcagcctgacatctgaagactctgcg
gtctatttctgtcagaggtgtgtgtactatagtaacttactgtgacttcgaltctggggcagcaggaccagggtaccgtctctgacagc
caggttcctcaactccaccctacccatctccctcaactccaccctacccatctccctcatgctgcacactggaactctctggggggaccgtcagttct
tctcttctcccccnaaaccaaggacacccctcatgatctccggaccctgaggtcacatgctgtgtgtgtgagcgtgagccacgaagacc
30 ctgaggtcaagttcaactgctacgtggagcgcgtggaggtgtgcatatgtccaaagacaagccggggaggaagcgtacacagcacgla
ccgtgtgtcagcgtctcaccgtctgtcaccaggagctggctgaatgtgcaaggaggtacaaggtcaaggtctccacaanaagccctccagc
cccatcgaagaanaactctccaaagccaaaggcagccccgaggaaccacaggtgtacacctgtcccccattccgggatgagctgacc
aagaaccaggtcagctgacctgctgtgtcaaaaggcttctatccagcagcatcgccgtggagtgggagagcaatgggcagccggaga

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acaaactaagaacccagctcccgtgctggactccgacgctccttctctacagcaagctaccgtggaacagagcaggtggcagc
aggggaaacgtcttctcatgctccgctgatgatgagctctgcacaaccactacacgagaagagcctcctctgctccgggtaaatgatct
aga

5 2H7 scFv IgAH IGG WT CH2CH3 protein sequence

mdfvqqlfllisavliargqivlsqspailsaspgkvtmtrassvsymhwyqqkpgsspkwiyapsn
lasgvparfsgsgstysltirveadaatyycqwsfnpptfgagtkleikdggsgggsgggssqaylqqsgaelvrpgasvk
msckasgyftfysnmhvwkqtrpqglewigaiypngdtsynqkfkqkaltlvdksslaymqllssltedsavfyfcarvvyysns
ywyfivwgtgttvtsdqpvpstptpsptpspcapellggpsvflfppkpkdltlmisrtpetvctvvdshdedpckfnwy
10 vdgvevhnaktkpreeqynstyrsvslvlhqdwlngkeykckvsnkalpapiektiskakgqprepqvyltppsrdeitknqvsit
clvkgyfypsdiaweensgqpennyktpvldsdgsfflyskltvdksrwqqgnvfscsvmhealnhhytqkslslspgk

NT

2H7 scFv IgAH IgACH2CH3 (2H7 scFv IgAhinge and IgA CH2 and CH3)

aagctgcgccatggatttcaagtcagattttcagcttcctgtaatacagtccttcagtcataattgccagagagaca
15 aattgtctctccagcttcacgaatcctgtctgcatctccagggaagaagtcacaatgacttgcaggccagctcaagtgtaagtacat
gcactggtacagcagaagccagatcctccccaacccctggatttatgcccatccaacctgctctctgagtcctgtctgcttcagtg
gcagtggtgtctgggacgtcttactctctcacaatcagcagaagtgaggctgaagatgctgccactattactgccagcagtggtgagtttaacc
caccacagctcgtgtcggaccaaagctggagctgaagatggcgtgtgctcggcgctgtgtgtgtatgtggagaggtggagagctc
20 ggcttatctacagcagtgctggggtgaagctgtgtgaggtggcctggcctcagtgaaagatgtcctgaaggtcttggtacatacattaccagt
acaatatgcactgggttaagcagacacctagacagggcctggaatggattggagctatttatcaggaatgtgtatattctcacaatcag
aagttcaaggcgcaaggccacactgactgtagacaaatcctccagcacagcctatctgcagctcagcagcctgacatctgaagactctgcg
gtctattctgtcaagagtggtgtactatagtaactcttactgtgactctgatgtctgggacagaggaccaggtcaccgtctctctgatca
ggcaggtctcccaactccacctacccatctccctcaactccactacccatctccctcatgctgccaccccgactgtcactgcaccagcc
25 ggccctcaggaagcctgtcttaggttcaagaagcagatctcagtcacactgaccggcctgagagatgctcagtggtgtacactcaactgg
acgcccctaagtgggaagagcgtgttcaaggaccacctgaccgtgacctgtgtgctgctacagcgtgtccaggtgtcctgccgggctgt
ggcagggcatggaacatgggaagacctcactgtcactgtcgtcctacccggagtcgaagaccccgtacaccggcaccctctcaaaatcc
ggaaacacatctccggccaggtgcacctgctgccgccgctcggggagagctggcctgaacagagctgtgtgacgtgacgtgctgtg
cagcgtggtctcagccccaagatgtgtgtgtcgtgctgctgcaggggtgcacagagagctgccccggagaaagtacctgacttgggacatcc
30 ggagaggaagccagccagggcaccaccactctgctgtgacagcctatctgcgcgtggcagccgagagctggaagaagggggacact
tctctgctgatgtgtggccacgagggccctgccgctggccttcacacagaagacacatgaccgttggcgggttaaaccacccatgtcaatg
tgtctgtgtcatgtggcgaggtgtgacggcacctgtctactgataatctaga

AA

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2H7 scFv IgAH IgACH2CH3 (2H7 scFv IgA hinge and IgA CH2 and CH3)

mdfivqvifslisasviiargqivlsqspailsaspgekvtmtcrassvsymhwyqqkpgsspkpwiypasn
lasgvparfsgsgtsysltisrveaadaatyccqwsfnptpfagtkleldggsgggsgsgssqaylqqsgaelvrpgasvk
msckasgytfsynmhvkvtpqrqlgwigaiypngdtsynqkfkgtatlvdkssstaimqlssltedsavyfcarvvyvns
5 ywyfdvwtgtttvssdqvpstptptpsptptpspcchprlsilhrpaedlllgseailctltglrdasgvtftwtpssgksavqgp
pdrldcgysvssvlpgaepwnhgtfcttaaypesktpiltalsksgntfrpevhlpppseelalnelvticlargfspdvlvrwlq
gsqelprekyltwasrqpsqgtttfvtlsilrvaedwkkgtfscmvghaelplafktidrlagkpthvnsvvmaevdgtcy

IgA hinge-CH2-CH3 (Human IgA tail, full length)

0 tgatcagccagttccctcaactcaccacccatccctcaactcaccacccatccctcatctgctgccaccc
cgactgtcactcaccgacggccctcagggacgtctcttaggtcagaagcgtacgtcgtcagcactgacggccgtcagagatgccc
tcagggtgacacctcaccctgagcgcctcaagtgggaagacgtctgtcaaggaccactgacccgtgacctgtgctgtctacagcgtgt
ccagtgctctgccggcgtgtgcgagccatggaacatgggaagacgtcactgtgctgctaccccgagtcacagaccccgctaa
ccgcccacctctcaaaalccggaaacacattccggccgaggtcaccgtgtgcgccgctgcggagagctggccctgaacgagct
15 ggtgacgtcagctgctggcacgtggcttcagccccaaggaatgtgctggttcgtgctgacgggggtcacagagctgcccgcgagga
agtacctgactgggcaccccgagagagccagccagggcaccaccactctcgtgtgaccagcactatgcgctggcagccgagga
ctggaagaagggggacacctctcctgcatggtgggcacgagggccctgccgtggccttcacacagaagaccatcgaccgctggcgg
gtaaacccaccatgtcaatgtgtctgtgatcgcggaggtggacggcactgtctactgataatctaga

IgA hinge-CH2-CH3 Protein sequence, (Human IgA tail, full length)

20 Dqpvpstptptpsptptpspcchprlsilhrpaedlllgseailctltglrdasgvtftwtpssgksavqgppdr
dlcgysvssvlpgaepwnhgtfcttaaypesktpiltalsksgntfrpevhlpppseelalnelvticlargfspdvlvrwlqsg
elprekyltwasrqpsqgtttfvtlsilrvaedwkkgtfscmvghaelplafktidrlagkpthvnsvvmaevdgtcy

Human J Chain:

25 agatctcaagaagatgaaggattgtcttctgtgacacaaatgtaagtgtgccggattacttcaggatcatccgttc
ttccgaagatctaatgaggacattgttgagagaacatccgaattatgttctctgaacacaggagaaatctctgatccaccctacc
attgagaaccagattgtgtaccattgtgtgacctcagctgtaaaaaatgtgatctacagaagtggagctggataatcatagattactgcta
30 cccaggaacatattctgtgatgaagacagtgctacagagacctgctacactatgacagaacaagtgtcacagctgtgtgccacctgcta
tatgtgtgtgagacaaatgttggaacagccttaaccocagatgctgctatctcgtactaatctaga

Human J Chain polypeptide

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rsqederivlvdnkckcaritsriirssedpnedivernirivplnnrenisdptslrtrfvyhlsdckkcdpsev
eldnqivtatqsnicdedsatetcytdmrkcytavplvyggetkmvetaltpdacyp

HUJCH5n1 (J chain 5' primer)

5 gtt gtt aga tct caa gaa gat gaa agg att gtt ctt

HUJCH3 (J chain 3' primer-antisense)

gtt gtt tct aga tta gtc agg ata gca ggc atc tgg

10 4 carboxy terminal amino acids deleted from IgA CH3
GTCY

IgAH IgAT4 Human IgA tail, truncated (3T1)-(missing last 4 amino acids from
carboxy terminus)

15 t g a t c a g c c a g t t c c c t c a a c t c c a c t a c c c c a t c c c t c a a c t c a c c t a c c c c a t c c c t c a t g c t g c c a c c c
c g a c t g t c a c t g c a c c g a c c g c c c t c g a g g a c c t g t c t t a g g t c a g a c g a t c c t c a c t g c a c a c t g a c c g g c c t g a g a g a l g c c
t c a g g t g t c a c c t t c a c c t g g a g c c c t c a a g t g g a a g a g c g c t g t t c a a g g a c c a c c t g a c c t g a c c c t g t g c t g c t a c a g c g t g t
c c a g t g t c t g c c g g c t g t g c c g a g c c a t g g a a c c a t g g g a a g a c c t t c a c t t g c a c t g c t g c c t a c c c g a g t c c a a g a c c c g c t a a
c c g c c a c c c t c a a a a t c c g g a a c a c a t t c c g c c c g a g g t c c a c t g c t g c c g c c g t c g g a g g a g c t g c c c t g a a c a g a c t
20 g g t g a c g c t g a c g t g c c t g c a c g t g g c t t c a g c c c a a g g a t g t g c t g t t c g c t g c a g g g t c a c a g a g a c t g c c c c g c g a g a
a g t a c c t g a c t t g g c a t c c c g g c a g g a c c c a g c c a g g g c a c c a c c a c c t t c g t g a c c a g a t a c t g c g c g t g c a g c c g a g a
c t g g a a g a a g g g g a c a c c t t c t c t a t g a t g t g g c c a g a g g c c c t g c c g t g g c c t t c a c a g a a g a c c a t c g a c c g t t g c g g
g t a a c c c a c c c a t g t a a t g t g t g t g t c a t g g c g g a g g t g g a c t g a t a a t c t a g a

25 IgAH IgAT4 Protein sequence:

Dqvpstptpsptpptspschprlsllhrpaledlllgseailtcltldrdsagvftwtpssgksavqgppdr
dlcgcysvssvlpgeacpwnhgktftctaaypesktptlatlsksntfrpevhllpppselaalnelvtlclargfspdvlvrwlqsg
elprekyltwasrqpsqgtttfavtsilrvaaedwkkgdtfscmvghalplafitqktidrlagkpthvnvsvmaevd

30 HUIGA3T1 (Oligo 3': to delete 4 amino acids at carboxy end of IgA CH3)
gtt gtt tct aga tta tca gtc cac etc cgc cat gac aac aga cac

HUIGA3T2: (oligo to delete 14 aa at end of IgA -T4)

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14 amino acids deleted from IgAH-T4 (so that total of 18 amino acids deleted from wild type IgA CH3)

PTHVNVSVVMAEVD

5

IgAH IgA-T18 (Human IgA Tail truncated, 3T2)

Tgactgacgagttccctcaactccactaccctacccatctccctcaactccactccctcatgctgccccc
ccgactgtcactgcaccgaccgcccctcaggacctgctcttaggttcagaagcgtctcactgtgcacactgaccggcctgagagatgc
ctcaggtgtcaacttccactggagcggccctcaagtgagggaagagcgtctgttcaaggaccacctgacctgtgacctctgtggtgcttacagcgtg
10 tccaggtgtcctggcgggctgtgtccgagccatgggaacatgggaagaccttcaactgtgacgtgctccatcccaggtccaagaccggccta
accggccacctctcaaaatccggaacacattccggcccgagggtccacctgctgcccggcctgctgggaaggagctggccctgaacgagat
gggtgacgtgacgtgctggcagcgtgtgttcagcccccaaggatgtgctgttgcgtgtgctgacgggttcacaggaagctgccccggcga
agtaacctgactgggcatocccggcaggaagccagccaggggcaccaccaccttgcgtgtgaccagcatactgcgcgtgtgcaagccgagga
ctgggaaggaagggggacacctctctctgcatgtgtggccacagggccctgcccgtgacctcacacagaagaccatcgaccgctggcggg
15 gtaaa

IgAH IgA-T18 Protein sequence:

dqpvpstptpsptpsptpspschprlslhpalcdlllgseailcttlglrdasvftwtvssgksavagppdrd
lccgysvssvlpgeapwnhgktftctaaypeskplitaltsksyntfrpevhllpppseelalneivltctclargfskpdvlvrwlqgsq
20 lprekyltwasrqepsqgtttfavtsilrvaedwkkgdtdfscmvghaelplafiqktidrlagk

NT

2H7 scFv IgAH IgAT18: (Human IgA Tail truncated, 3T2.)

aagctgcgcccatggattttcaagtgcagatttcaagcttctgtcaatcagtgtctcaagtcaattgaccagaggaca
aattgttctccaggtctccagcaatctgtctgcatctccaggggagagaaggtcacaatgacttcagggccagctcaagtgtaagtatcat
gcactggtagcagcagaagggcaggatctcccccaaaccttgattatgcccatccaacctggcttctggaggtccctgctgtctcaagt
gcagtggtgtctgggaaccttactctctcacaatcagcagagtggaaggctgaagatgctgcaccttattatcagcagagtgaggttttaacc
caccacaggttgcgtgctggaccaaagctggagctgaaagatggcggctgctcggcggtggttgatctggaggaagggtggggctctca
30 ggcttatctacagcagctgtgggctgagctgtgagggcctggggcctcagtgagaagatgtctcgaaggctctggctacacatttaccagtt
acaataigcactgtgttaagcagacacctagacaggcctgggaatggattggagctattatccaggaagtgtgatacttctcaaatcag
aagttcaagggaaggccacactgactgtagacaaalccctccagcagagcctacatgcagctcagcagcctgacatctgaaagctctgcg
gtctatttctgtgcaagagtggtgtactatagtaacttctactggtacttcgatgtctggggcacagggaaccaggtcaccgtctctctgatca

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gccaagttccctcaactccaccctaccccatctccctcaactccactaccccatctccctcatgctgccacccccgactgtcactgcaccgacc
ggccctcagaggacctgctcttaggttcagaaagcagatcctcagctgcacactgaccggcctgagagatgctcaggtgtcaccttcacctgg
acgccctcaagtgagggaagcgcgtgtcaaggaccacctgacctgtgtgctgctacagcgtgtcaggtgtcctgcccggctgt
gcccgaagccatggaacctgggaagaccttcacttgctgctgcttaccggagtcgaagaccccgtaaccgccacctctcaaaatcc
5 ggaacacattccggcccgaagtccacctgctgcccggcgcgtcggaggagctggccctgaacgagctgtgtagcgtgacgtgctgg
cacgtgcttcagcccaagaaagtgtgctgctgctggctgcaggggtcacaggagctgccccgggaaggtacctgacttgggcatccc
ggcaggagcccgacggcaccaccacctctgctgtgaccagatactgcgcgtggcagccggagactggaagaagggggacacct
tctctgcatggtgggcacgagccctgccgtggccttcacagaagacctgaccgcttggcgggtaaa

0

AA:

2H7 scFv IgAH IgAT18:

mdfqvqifslilisasviiaqgqivlsqpailsaspgekvtmtrasssvsymhwyqqkpgsspkwiyapn
lasgvparfsgsgtsylsitrvaedaatyycqwsfnppftfgagtkleldggsgggsgggssqaylqsgaelvrpagsvk
msckasgyftfynmhwwktpqrqglewigaiypngdtsynqkfkqkalitvdksstaymqllssedsavvfcarvvyysns
15 ywyfdvwgtgtvtssdqpvstppstppstppstpschprlsilhrpaeldllgseailcttglrdasgvtftwpsggksavqgp
pdrldcgysvssvlpgeapwnhgktftctaaypesktpitatlslsgntfpevhllpppsealnelvitclargfspkdvlvrwlq
gsqelprekyltwasrqepsqglttfavtsilrvaedwkkgdftscmvghealplafktidriagk

20

CTLA-4 IgG WTH WTCH2CH3 (Human-oncoMLP-CTLA4EC-hIgGWT)

Nucleotide sequence:

gcaacctacatgatgggggaatgagttgaccttcctagatgattccatctgcacgggacacctccagtggaatcaagtg
aacctcaactccaaggactgagggccatggagacgggagctcactcgaagggtgagctcatgtaccacgcacatactacctgggc
ataggcaacggaaccagatttatgtatgatccagaaccgtgccagattctgatcaaccacaaactgttgacaaaactcacacatgccca
25 ccgtgccagcaactgaactctgggggacccgtcagctcttctctcccccacaaacccaaggacacctcatgtatctccggaccctg
aggtcactgctggtgtggtgagcgtgagccacgaagacctgaagggtcaagttcaactgtacgtggcagcggcgtggaggtgataaagcc
aagacaaaagccgcgggaggaagcgtacaacagcacgtaccgtgtgtgacagcgtctaccgtctgcaccaggaactggtgtaaggga
aggagtgcaagtgcaaggtctcnacaaagccctccagcccccacatcgagaacaatctccaaaggccaaggcagccccgagaacc
acaaggtgtacacctgccccatcccggatgagctgaaccaagaacggatcagcctgacctgctgctgcaaaaggcttctatccagcga
30 catcgccgtggagtgaggagagcaatggcgagccggagaacaaactacaagaccagcctcccgctgctgacctccgacggctcctcttcc
tctacagcaagctcaccgtggacaaagcagcaggtggcagcaggggaacgtcttctcatgctccgtgatcatgagcgtctgcacaaacct
acaacgcaagaagcgtctcctctgctctccgggtaaatga

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CTLA-4 IgG WITH WTCH2CH3 Protein sequence:

mgvlltqrllslvlllfpmsmamhvaqpavvlssrgiasfvceyaspgkatevrvtlrqadsqvtevc
 5 tymmgneltfddisictgtssgnqvnltiqglramdtglyickvelmypyppylgigngtqiyvidpepcpdsdpkscdkthtppc
 papellggpsvflfppkpkdtlmisrtpevtcvvvdshedpevkfnwyvdgvevhnaktkpreeqynstyrsvsvltvlhqdwl
 gkeykckvsnkalpapielktiskakgqprepvytlppsrdeitknqvsitclvkgfypsdiavewesngqpennyktppvlidsd
 fflyskltvdksrwqqgnvfscsvmhleahhnytklsislspgk

Human OncoM leader Peptide+CTLA4 EC (BcII)

Atgggggtactgctcacacagaggacgctgctcagctgtgtccttgccactcctgtttcgaagcatggcgaagcatggc
 0 aatgcacgtggccacgctgctgtggtactggtccagcagccgagcgcacgtccagctttgtgtgagtatgcatctccaggcaangccact
 gaggctccgggtgacagtgtctcgacaggtgacagccaggtgactgaagtctgtgcggcaacctacatgatgggaatgattgaccttc
 ctatagatgattccatctgcacggcgacctccagtggaatacgaatgaacctcactatccaaaggactggggccatggacacgggactctaca
 tctcgaaggtggagctcatgtacccaccgccatactacctggcgataggcaacggcaaccagatttatgtaattgatccagancctgtgcc
 agattctgatcaa

Human OncoM leader Peptide+CTLA4 EC Peptide sequence:

mgvlltqrllslvlllfpmsmamhvaqpavvlssrgiasfvceyaspgkatevrvtlrqadsqvtevc
 10 tymmgneltfddisictgtssgnqvnltiqglramdtglyickvelmypyppylgigngtqiyvidpepcpdsdpk

Human OncoM leader peptide nucleotide

atgggggtactgctcacacagaggacgctgctcagctgtgtccttgccactcctgtttcgaagcatggcgaagcatg

Human OncoM leader peptide sequence:

Mgvlltqrllslvlllfpms

NT

Human CTLA4 EC (no LP)

Gcaatgcacgtggccacgctgctgtggtactggccagcagccgagcgcacgtccagctttgtgtgagtatgcat
 30 ctccaggcaaaagccactgaggtccgggtgacagtgtctggcaggtgacagccaggtgactgaagctgtgcggcaacctacatgacg
 gggaatgattgaccttctgattgattcatctgcacggcgacctccagtggaatacgaatgaacctcactatccaaaggactgaggccat
 ggacacgggactctacatctgcaaggtggagctcatgtacccaccgccatactacctggcgataggcaacggcaaccagatttatgtaatt
 gatccagaaccgtgccagattct

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AA

Human CTLA4 EC (no LP)

Amhvaqpavvlassrgiasfveyaspkatevrvvtrqadsqvteveaatymtgneltfddsiotgssngn
vnltiqglramdtglyickvelmypppyylgigngtqiyvidpepcpds

5

NT

Human CTLA4 IgG MTH (SSS) MTCH2CH3

Atgggggtactgctcacagaggacgctgctcagctggtcttgcactctgtttccaagcatggcgagcatggc
aatgcacgtggccacgctgctgtgtactggtccagcagccgagcagcagcttctgtgtgagatgatcatctcaggcaaaagccat
10 gaggtccgggtgacagctgttcggcagcgtgacagccaaggtgactgaagctctgtgcggcaactcatatgatgggaatgagttgaccttc
ctagatgatccatctgcacgggacacctcagtggaatcaagtgaaacctactatccaaggactgagggccatggacacgggactctaca
tctgcaagggtgagctcatgtacccaccgccatactacgtggcagcgaacggacccagatttatgtaattatccagacccgtgcc
agattctgatcaacccaatctttgacaaaactcacacatccccaccgtccccagcaactgaactcctgggggagtcagctctctctt
cccccaaaacccaaggacacacctcatgatctcccggacccctgaggctacatgctgtgtgtgtgagctgagccacgaagacctgag
15 gtcaagttcaactgtacgtggagcggcgtggaggtgcataatgccaagacaaagccgctgggagagcagtaacacagcagctaccgtg
tggtcagctgctcaccgtctcgcaccagcagctggctgaatggcaaggagtaacaagtgcaaggtctccaacaaagcctccagccccc
atcgagaanaacatctccaagccaaggcagccccgagaccacaggtgtgacacctgcccccatcccgggagtgagctgaccaa
accaggtcagcctgacctgctgtgtcaaaagcttctatccacgcacatgcctgtggagtgaggagagcaatgggacgcccggagaa
ctacaagaccacgcctcccgctgctggactccgacggctcttctctacagcaagctcaccgtggacaaagcaggtgagcagcagggg
20 gaacgtctctcatgctcctggtgatgaggtcctgcacaaccactacacgcagaaagacgtctcctctgctccgggtaaatga

AA

Human CTLA4 IgG MTH (SSS) MTCH2CH3

Mgvlltqrlslslvllfpmasmamhvaqpavvlassrgiasfveyaspkatevrvvtrqadsqvtevea
25 atymmgneftfddsiotgssngnvnltiqglramdtglyickvelmypppyylgigngtqiyvidpepcpdsdpkssdkhtstpp
spapellgssvflfpkpkdtlmsrtpevicvvvdshedpevkfnwvdgvevhnaktpreqynstvrsvvltvlhqdwl
gkeyckcvsknalpapektiskakgqprepqvylppsrdeitknqvsitclvkgfyfysdiaewesngqpenntktpvldsdgs
fflyskltvdksrwqggnvfscsvmhlnhytkslslspgk

30

CTLA-4 IgA/IgG MTH (SSS) MTCH2CH3 (Human-oncoMLP-CTLA4EC-IgA)

Nucleotide sequence:

WO 2005/037989

PCT/US2003/024918

atgggggtactgctcacacagaggagcgtgctcagctggctcctcactcctgtttcaagcatggcggagcatggc
 aatgacagctggccacagcctgctgtgtgtactgtgcccagcagccgaggcagcagccagcctttgtgtgtgagatgacatctccaggcaagccact
 gaggtccgggtgacagctgcttcggcagcgtgacagccaggtgactgaagctgtgtgtgccaacctacatgatggggaatgagttgaccttc
 ctatgagatgattccatctgacggcagcctccagtggaatcaagtgaacctcactatccaaaggactgagggccatgagacacgggactctctaca
 5 tctgcaagggtggagctcatgacccaccgccatactacctggcataggcaacgggaaccagattatgtaattgacccaagaccgtggcc
 agattctgatcagccagttccctcaactcacctaccccatctccctcaactccactaccccatctccctcatgctgccaccccgactgtca
 ctgcaccgaccggccctcaggacgtgctcttaggttcagaagcgatcctcagctgacacgtgacccgctgagagatgctcaggtgtc
 accttcacctggagccctcaagtgagggaagcgtgttcaaggacacctgaccgtgacctctgtggctgctacagcgtgtccaggtgtc
 tgcgggctgtggcggccatggaacatgggaagaccttactgtgactgctgctaccccgaggtccaagaccgccgctaaccggcacc
 0 ctctcaaatccggaaacacattccggccgaggtccacctgctgcccggcgtcggaggagctggccctgaacgagctgtgagct
 gacgtgctgtggcagctggcttcagcccaaggatgtgctgttgcctgctgcaggggtcacaggagctgccccgcggaagatctga
 ctgggcatcccgaggagccagccagggcaccacccacttgcgtgtgacagcatactgctgctgacggccgagggactgggaagaa
 ggaggacacctctcctcatggtggcgcacagggccctgcccgtgctgcttcacacagaagaccatgaccgcttgagggtgaaccaca
 cccatgtcaatgtgtctgtgtcatggcggaggtggacggcaccctgctactataatctaga

5

CTLA-4 IgAH IgACH2CH3 Protein sequence:

mgvlltqrllslvlallfpmasmamhvaqpavvllasrgiasfvcyspgkagctevrvlvrqadsqvtevcaa
 tynrmgneltfddtsictgssgnqvnltiqglramdtglyickvelmypppyylgigngtiqiyvidpepcpsdqpvpstptpspt
 pptpspcschprlsihrpaledlllgseailtcltglrdasgvtflwpsgksavqgppdrldlcygssvslpagaepwnhkgkftcta
 10 aypeskipltatlsksgntfpevhllppseelalnelvtitclargfspkdvlvrwlqgsqelprekyltwasrpepsqgtttfvatsilrva
 acclwkkgtfscmvghcalplafktidrlagkpthvnsvvmaevdgtcy

CTLA-4 IgAH IgA-T4 (Human-oncoMLP-CTLA4EC-IgA3T1)

Nucleotide sequence:

atgggggtactgctcacacagaggagcgtgctcagctgtgctcctcactcctgtttcaagcatggcggagcatggc
 aatgacagctggccacagcctgctgtgtgtactgtgcccagcagccgaggcagcagccagcctttgtgtgtgagatgacatctccaggcaagccact
 gaggtccgggtgacagctgcttcggcagggctgacagccaggtgactgaagctgtgtgtgccaacctacatgatggggaatgagttgaccttc
 ctatgagatgattccatctgacggcagcctccagtgaatcaatgaacctcactatccaaaggactgagggccatgagacacgggactctaca
 25 tctgcaagggtggagctcatgacccaccgccatactacctggcataggcaacgggaaccagattatgtaattgacccaagaccgtggcc
 agattctgatcagccagttccctcaactcacctaccccatctccctcaactccactaccccatctccctcatgctgccaccccgactgtca
 ctgcaccgaccggccctcaggacgtgctcttaggttcagaagcgatcctcagctgacacatgaccggcctgagagatgctcaggtgtc
 30 accttcacctggagccctcaagtgagggaagcgtgttcaaggaccacctgacctgacctgtgtgtgctctacagctgtctcaggtgtcc
 tgcgggctgtggcggccatggaacatgggaagaccttactgtgactgctgctaccccgagtcgaagcccgctcaaccggcacc

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5

10

15

ccctgaactctctgggggggagtcagctctctctctcccccacaaaccaaagacacctcatgatctccggaccacct
gagggtcacatgctggtggtgctgagcagagccacgaagacctgagggtcaagttcaactgtgacgtgagccggctggaggtgcataatgc
caagacaaaccggcgggaggagcagtacaacagcagctaccgtgtggtagctgctctaccgtctgcacaggagctgctggaatgac
aaggaggtacaagtgcgaaggtctccacaagaagccctccagcccccacgagaaaaccatcctccaaaagccaaag

pellggssvflfpkpkdtlmsrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpreeqynstyrvvs
vltvlhqdwlngkeykckvsnkalpapiektiskak

PAPELLGGSS

147

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hIgE3stop

gtt gtt tct aga tta act ttt acc ggg att tac aga cac cgc tgc ctg g

- 5 hIgE3BB (leaves an open reading frame at end of gene to read into transmembrane and cytoplasmic tail domain attached at either the BamHI or SfuI sites)

gtt gtt ttc gaa gga tcc gct tta cgg gga ttt aca gac acc gct cgc tgg

10

NT

human IgE Fc (CH2-CH3-CH4) ORF:

- tgatcacgtctgctccagggaactcaccgccaccgtgaagatcttacagtcgtcgcgagggcgggcac
ttccccccgaccatccagctcctgtgctcctgtctctgggtacaccccaaggactatcaatcacctggctggaggacgggcaggtcatgg
15 acgtggacttgccaccgctctaccacgcaggagggtgagctggcctccacacaaaggcagctcacctcagccaggaagcacttgctg
tcagaccgcacctacacctggccagggtcacctatcaagggtcacactttgaggacagcaccaagaagtgtgcagattccaaccggaggg
gtgaggcgcctacctaaagccggccagcccgttcgacctgttcatccgcaagtcgccacgaltcacctgtctgtgggtggacctggcacc
agcaagggggacctggaacctgacctgtcccgggccagtgaggcctgtgaaccactcaccagaaaaggaggaagagcagcgcaant
ggcacgttaacctgtcacgtccacctgcccgtgggaccccgagactggtatcgagggggagacctaccagtgacgggtgaaccaccccc
20 acctgcccaggccctcatgctgggtccacgaccaagaccagcggcccgcgtgtctgccccggaagtctatgctgttgcgacggcgagtg
ggcgggggagccgggacacggcgcacctcgcctgctgatccagaacttcagcctgagacatctcgtgacgtggctgcacaaagg
gtgcaacctccggagcggccgacacgacgacgagcccgccagaccaaagggtccgctctctgtcttcacggcctgaggtga
ccagggccgaatgggagcagaagatgagttcatctgcctgtgacgtccatgagcagcgagccctcacagaccgtccagcgagcgt
gtctgtaaatcccggtaaaggcgatccttcgaa

25

AA

human IgE Fc (CH2-CH3-CH4) ORF:

- dhwcsrdftppvtkilqsscdggghfppitqllclvsytpgtinitwledgqvmvdvlstastqegelasqsel
30 sqkhwlslrtyctqvtqgthfedstkkcadsnprgvsaylsrspfdlfrksptctlvldlpskgtnvlntrsagkpxvnhstrkee
kqmgltvltstlpvgtrdwiegetyqcrvthphlpralmrstktsgpraapevyafatpewpwsrdlctlaclqnfmpedisvqwh
nevqlpdrharistqprktksgffvlsrlevtraeweqkdeficravheaaspsqtvqrvsvnpkgadps

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PTC/US2003/024918

IFhIgGwtBcl5

ggt gtt tga tca gga gcc caa atc ttg tga caa aac tca cac atg ccc acc gtg ccc agc acc
(63 mer)

5

hIgGWT3xba
ggt gtt tot aga tca ttt acc cgg aga cag gga gag gct ctg ctg gta g

HuIgGMHWC (sense, 5' primer for mutating wild type hinge CCC to mutant

10 SSS'

ggt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca cgg tcc cca gca cct gaa
ctc ctg ggt gga cgg tca gtc ttc c

NT

15

1D8 VH

caggctgagctgaaggaggcaggacctggcctggtgcaaccgacacagacctgtccctccatgcactgtctctg
gggtctcattaacccagcgtggtgtacactggattcgacagcctccaggaaagggtctggaatggatgggaataatattattatgatggc
acaagattataattcagcaattaatccagactgagcatcagcaggcgcacacctccaaggccaagttttctnaaaatcaacagctgtcgaact
gatgacacagccatgtattactgtgccagaatccacttgattactggggccaaggagtcagtggtcacagctctcctt

20

AA

1D8 VH (no leader)

qvqlkeagpglvqptqtllstctvsgfsltsdgvhwirppgkglewmgiyydggtdynsaiksrslsrdtsks
qvflkinslqtdtdamyycarihfdywgqvmvtvss

25

NT

1D8 VL (no leader)

gacattgtgctcactcagctctocacaacccatagctgcatctccaggggagaggtccaccatccctgcccgtgccag
ctccagtgtaagtacatgtactgtgaccagcagaagtcaggcgccctccctaaactctggatttatgacacatccagctgcttctggagtt
30 ccaaatcgctcagtggcagtggtctgggacctctattctctcgcaatcaacaccatgggagctgaagalgtgccattattactgtcagc
agtggagtagtactccgctcacgttcgggtctgggaccaagctggagatcaaacgg

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AA

1D8 VL

divltqspitiaaspgekvtitcrassvssymwywyqqksgasplwydtsklasgvpnrfsgsgtsyslaint
metedaatyycqwsstpltfsggkcleikr

5

NT

1D8 scFv

aagcctatggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacatt
10 gtgctcactcagcttcacaacacatagctgcattctcaggggagaaaggtcacatcacctgccgtgccagctcagtgtaagttacatgta
ctgtgaccagcagaagtcaggcgccctccctaaactctgatttatgacacatccaagctggctctgtgaggtccaaatgcgttcagtgca
gtgggtctgggacctctattctctcgaatcaacacacatggaagactgaagatgctgcacattactgtcagcagtggaagtactaccgct
cacgttcgggtctgggaccagctggagatcaaacgggggtggcgtgctcgggcgggtggtgggtggcggcggaatcagggg
cagctgaaggaggcagggacctggcctgtgtgaaccgacacagaccctgtccctcacatgactgtctctgggttctcatiaaaccagcga
15 gtgtacactggattcgaagccctcaggaagggtctgtgaatggaatgggaataatattatgatggagacagattataatcagcaatta
aatccagactgagcatcagcagggaacacctcaagagccaagttttctanaaatcaacagctgcgaactgatgacacagccatgattac
tgtgccagaatcaatttgattactggggccaaggagtcagtggtcacagctctcctctgatca

AA

1D8 scFv

mdfqvqfsslliasvimrsgvdivltqspitiaaspgekvtitcrassvssymwywyqqksgasplwydtskl
asgvpnrfsgsgtsyslaintmetedaatyycqwsstpltfsggkcleikrgggsgsgsgsgsgsgsqvqlkagpglvqptqlsl
ctvsgfsltsdgvhwrppgkglewmgiiyydgdtdynsaikrslsrdtsksqvfklinslqtdtdamyycaihfdywgqgv
vtvss

25

NT

1D8 scFv IgG WTH WICH2CH3

aagcctatggattttcaagtcagattttcagcttcctgctaatacagtgcttcagtcataatgtccagaggagtcgacatt
30 gtgctcactcagcttcacaacacatagctgcattctcaggggagaaaggtcacatcacctgccgtgccagctcagtgtaagttacatgta
ctgtgaccagcagaagtcaggcgccctccctaaactctgatttatgacacatccaagctggctctgtgaggtccaaatgcgttcagtgca
gtgggtctgggacctctattctctcgaatcaacacacatggaagactgaagatgctgcacattactgtcagcagtggaagtactaccgct
cacgttcgggtctgggaccagctggagatcaaacgggggtggcgtgctcgggcgggtggtgggtggcggcggaatcaggtg

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PCT/US2003/024918

5

1D8 scFv IgG MTH MTCH2CH3-CD80

15

1D8 scFv IgG WTH WTCH2CH3-CD80

25

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PCT/US2003/024918

ccaaacc caagacaccctcatctccggacccctgaggtcacatgctggtgggtgagcgtgagccacgaagaccctgaggtcaa
gttcaactgtgtacgtggacggcgtggaggtgcataafgccaaagcaaacccggggagagcagtaacaacagcacgtaccgtgtggtc
agcgtcctcaccgtcctgcaccaggactggctgaatggcaaggagtaacaaggtccaaggtctccaaagccctccagcccccacgca
gaaacaa.tctccaaaggccaaggcgagcccgagaaccacaggtgtacaccctgcccccatccgggatgagctgaccaagaacca
5 ggtcagcc tgcactgcctgtcacaaggcttctatccagcgacatgcctggtgaggtggagagcaatggggacccggagacaactaca
agaccagcgctccgtgctggactcgagggctctcttctctctacagcaagctcaccgtggacaagagcaggtggcagcaggggaac
gtcttctcatgctccgtgatgcatgaggtctgcacaaccactacagcagaagagcctctccctgctccgggtaaatgatctaga

AA

10 Anti human CD3 scFv WTH WTCH2CH3

Mdfqvqifsfllisasvimsrgvdiqmtqtsslsaslgdrvtircasqdirnylnwyqqkpdgtvkillytsrlh
sgvpsrfsrgsgsgtdysltianlqpediatyfcqqnltlpwtfggggtklvtkrelggggsgggsgggsgsi devqlqsgpelvkpgas
msckasgyrftgyivnwlkqshgknlewiglinpyklttynqkfkkgkatltvdksstsaymellsdtsedavyyarsgyygsd
wyfdwvgagttvtvssdqepksdktthtppcapellggpsvflfpkpkdltlmsrtpvtcvvvdshedpevkfnwyvdgv
15 evhnahtktpreeqynstyrvvsvltvlhqdwingkeykckvsnkalpapiektiskakgqprepqvlytpprsdeltnqvsiltelvk
gfypsdia vewesngqpennyktpvldsgsfllysklvtksrwqqgnvfscsvmhahlnhytqkslslspgk

NT

20 2H7-antiCD40 scFv MTH (SSS) MTCH2WTCH3

2h7-40.2.220Ig + restriction sites

aagcttgccgccatggaatttcaagtcgaattttcagcttctgtctaatacagtcgttcacataatgccagaggaca
aatgtctctccacgtctccagcaatctgtctgcatctccaggggagaaggtcaaatgacttcaggccagctcaagtgtaattacat
gcactgtgtaccagcagaagccagatcctccccaaccctggaatttatgccctccaacctggcttctggagtcctgtcgtctcagtg
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caccacgcttggtgctgggnccaagctggagctgaagggtggcgtgctcggcgtgtgtgctatcgagaggtgtggagctctca
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atcacatgagtgtccaaaggtctctcatcaaatatgcttccattccatctctggatccctccaggttcagtggtgagcaggggtcagatt

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cctgagctgaaagaagcctggagagacagtcaggatctctgcaaggctctgggtatgcttcaaacactctggaatgcagtggtgcaag
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5 cttctcttggaaaccttgccaacactgcataattacagatnagcaacctcaagaatgaggacacggctacgtattctgtgtgatccggg
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cacacatcccccacgtccccagcactgaaactctggggggatgacgtacttctcttcccccaaaacccaaaggacacctcatgatctc
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10 gctgaatggcaaggaggtacaaagtcaggctctccaacaaagccctccagcccccacgaganaaaacatctcaaaagccaagggcag
ccccgagaaccacaggtgtacacccctgccccatccgggatgagctgaccaagaaccagggtcagctgacctgctggtcaaaaggctt
ctatccacgcgacatcgccgtggagtgaggagacaaatgggcagccggagaaacactacaagaccacgctccctgctgactccgac
ggctctcttctctacagcaagctaccgtggacaagagcaggtggcagcaggggaacgtctctctatgctcctgcatgatgaggtct
gcacaacactacacgcagaagagcctctccctgctcgggtaaatgacataga

15

AA

2H7-antiCD40 scFv MTH (SSS) MTCH2 WTCH3

2H7-40.2.220Ig

mdfqvqifslisaviiaqgqivlsqspailsaspgekvlmtcrasssvsynhwyqqkpgsspkpwiyapsn
20 lasgvparfsgsgstysltisrveadaatyycqawsfnptptgagtklelkgggsgggsgggssqaylqqsgaelvrpgasvk
msckasgyrtfssnmhwwkqtpqrglewigaipngdtsynqkfkgtatltvdksstaysmqslsdsedavycarvvyysns
ywyfdvwtggtvtvssdqsneekakeekakeaksnsdvdivltqspatlsvtpgdrvlscrasqsidsylhwyqkshspsrlli
kyashsigsipsrfsrgsgsddflsinsvepedvgiyycqhgshspwtfgggtkleikregggsgggsgggsgqilvqsgpelkk
pgetvriscasgyafittgmqwqempgkglkwigwintplwsakicrrlqgrfalsletsantaylqisnlkdedtatyfcvrsng
25 nydlayfaywggqtlvtvsdqpkkdkthtspspapellgssvflfpkpdkdlmsrtpevtcvvdvshedpevkfnwydvg
vevhnaktkpreeqynstvrsvltvlhqdwlngkeykckvksnkalpapiektiskakgqprepvtylppsdeltknqvsitclv
kgfypsdiaevewesngpennyktppvldsdgsfflyskltvdksrwqgnvfscsymhealhnhytqslslspgk

30

NT

5B9 VH (includes the VH leader peptide)

atggctgtcttggggctgctcttctgctgtgacatttcaagctgtgtcctatccagggtgcagctgaagcagtcag
gacctggcctatgtagctctccacagagcctgtccatcactgcacagtcctgtgttctcattaacactatgatgtgacactgggttcacca
gtctccagcgaagggtctgagtgctggagtgatagtggtgggaatcacacagactaataatgcagcttctatccagactgacatca

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ccaaggacgattccaaagagccaagttttcttfaaaatgaacagctctgcaacctaatgacacagccatttattctgcccagaaatgggggtg
ataactacccttattacatgctctgactactgggtcagggaacctcagtcaccgtctctca

5B9 VH missing the leader:

5 cagggtgcagctgaagcagtcaggacctggcctagtgcagtcctcacagagccctgtccatcacctgcacagtctctg
gtttctcatfaactaccatgctgtacactgggttcgccagctctccaggaaagggtctggagtgctgggaagtataaggagtggtggaatca
cagactataatgcagctttcatatocagactgagcatcaccaaggacgattccaagngccaagttttcttfaaaatgaacagctctgcaacctaa
tgacacagccattattactgtgccagaaatgggggtgataactacccttattactatgctatgactactgggtcagggaacctcagtcacc
gtctctca

10

AA

5B9 VH (includes leader peptide)

MAVLGLLFLVTFPSCVLSQVQLKQSGPGLVQSSQSL SITCTVSGFSLTTY
AVHWVRQSPGKGLEWLGVIWWSGGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDT
15 AIYYCARNGGDNYPIYYAMDYWGQGTSTVTVSS

5B9 VH no leader peptide

QVQLKQSGPGLVQSSQSL SITCTVSGFSLTTYAVHWVRQSPGKGLEWLG
VIWWSGGITDYNAAFISRLSITKDDSKSQVFFKMNSLQPNDTAIYYCARNGGDNYPIYYA
20 MDYWGQGTSTVTVSS

NT

5B9 VL

atgagggttctctgctcagcttctgggagctgctgtgctctggaacctgcagtcagatattgtagacgcagg
25 ctgcattctccaatccagtcactcttggaaacatcagcttccatctcctgcaggtctagtaagagcttccatagatattggcatcactattttaga
tttggtatctgcagaaaggccaggccagctctcctcctcctgatttaccagatgtccaaacctgcctcagagagtcocagacagggttcagtagcag
tgggtcagggaactgatttcacactgaagaatcagcagagtgaggctgaggatgtgggtgtttattactgtgctcaaaatctagaactccgct
cacgttctggctgctggaccagagctggagctgaaacgg

30

AA

5B9 VL

WO 2005/037989

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MRFSAQLLGLLVLPWPGSTADIVMTQAAFSNPVTLTGTSASISCRSSKSLHH
SNGITYLYWYLQKPGQSPQLLIYQMSNLAGVDPDRFSSSGSGTDFTLRISRVEAEDVGV
YYCAQNLELPLTFGAGTKLELKR

5 NT
5B9 scFv

aagcttgcggccatgaggttctctgctcagcttctgggctgctgtgctctggaatccctggaatccactgcagatattgt
gatgacgcagcgtgcattctccaatccagtcactcttggaacatcagcttccatctctgcaggtctagtaagagctctccatagataatggc
atcaacttattgtattggtatctgcagaagccagccagctcctcagctcctgattatcagatgtccaacctgctcaggatccacagacg
10 gttcagtagcagtggtgacgaactgattcacactgagaatcagcagagtgaggtgaggtatgggtgtttattactgtgctcaaaatct
agaacttccgctcagcttgcgtgggaccaagctggagctgaaacgggggtggcgctggcggtgggtgggtcgggtggcgccg
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5B9 scFv
20 MRFSAQLLGLLVLPWPGSTADIVMTQAAFSNPVTLTGTSASISCRSSKSLHH
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25 NT
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15

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5B9 scFv-hmIgG1-hCD80

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20 YYCAQNLLEPLTFGAGTKLELKRGGGSGGGSGGGSSQVQLKQSGPLVQSSQSLSI
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30

NT

2e12 scFv WTH CH2 CH3 (2e12 scFv-WithIgG-CD80)

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20 2e12 scFv WTH CH2 CH3 2e12 scFv-WtIgG-CD80
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NT
30 2H7-human IgE Fc (CH2-CH3-CH4)
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20 AA

2H7 scFv IgE (CH2-CH3-CH4)

mdfqvqifslisaviiargqivlsqpailsaspgekvtmtcrasssvsynhwyqqkpgsskpwpiwapsn
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30 NT

2H7 scFv MH (SSS) MCH2WTCH3

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AA

15 **5B9 scFv MTHWTC2CH3**
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TCTVSGFSLTTYAVHWVRQSPGKLEWLGVIWSSGITDYNAAFISRLSITKDDSKSQVF
20 FKMNSLQPNDDTAIYYCARNGGDNYPIYYAMDYWGQGTSTVTVSSDQEPKSSDKTHTSP
PSPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN
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FFLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK
25

Human IgG1 hinge mutations

30 **2H7 scFv- MTH (CSS) WTC2CH3**

Nucleotide:

aagcttgccgccatggtatitcaagtgcaagtttctgcttaacagtgcttcagtcataattgccagaggaca
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2H7 scFv- MTH (CSS) WTCH2CH3 protein:

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25 2H7 scFv- MTH (SCS) WTCH2CH3:

Nucleotide:

aagctggccgcctgagatttcaagtgcaagatttcagctcctgctaatacagtgcttcagtcataatgccagaggaca
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2H7 scFv- MTH (SCS) WTCH2CH3 Protein:

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20

2H7 scFv- MTH (SSC) WTCH2CH3:

Nucleotide:

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2H7 scFv- MTH (SSC) WTCH2CH3 Protein:

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HIgGMHcys1

20 gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tg

HIgGMHcys2

gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tct oca ccg tgc

HIgGMHcys3

25 gtt gtt gat cag gag ccc aaa tct tgt gac aaa act cac aca tgt oca ccg tcc oca gca cct

NT

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10 AA

2H7 scFv MTH (SSS) WTCH2MTH3Y405

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5 2H7 scFv MTH (SSS) WTCH2MTH3A407
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caactgtgtacgtggacggcgtggaggtgacataatgccaagacaaagccggggaaggagcagtacaaagcagctgacgtgtgtgacg
gtctcaccgtctgcaccagagactgctgaatgcaaggagtagcaaggtctcacaacaagccctccagcccccacacgagaa
aacaactctcaaaagccaaaggcagcccccgaaccacaggtgtacaccctgccccatcccggatgagctgaccaaagaacaggctc
agcctgacctgctgctgaaggtctctatccagcgacatcgccgtggaagtggagagcaatggcagccggagagaacaactacaaga
25 ccacgcctccgtgctgactccagcggctctctctctctacagcaagctcaccgtgacaaagcaggtggcagcaggggaacgct
tctcatgctccgtgatgcagggctctgcacaaccactacacagcagaagagccctcctgtctccgggtaaatgatctaga

AA

2H7 scFv MTH (SCC) WTCH2CH3

30 mdfqvqifslisaviargqivlsqspailsaspgekvtmtcrassvsymhwyqqkpgsspkpwiyapsn
lasgvparfsgsgstysltsirveadaatyycqwsfnptfagtklelkdggsgggsgggssqaylqqsgaelvrpgasvk
msckasgyftsy nmhvwkqprqglewigaiypngdtsynqkfkgtatlvdksstaymqllsitedsavyfcarvvyvns
ywyfdvwtgttvtssdqepkssdkhtcpapapellggpsvflfppkpkdtlmsirtpetvctvvdvshdepvkfnwyvdgv

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2H7 scFv MTH (CCS) WTCH2CH3

aagcttgccgccattttcaagtgcagattttcagcttcctgctaaticagcttccataatgccaggagaca
aatgtctctccagctctcagcaatcctgtctgcactccaggaggagaaggtcacaaatgacttgcaggccagctcaagtgaagtacat
gcacttggtaccagcagaagccaggatcctccccaaccctcgatttatccccccaacctggtctctgagctccctgctcgtcctaagtg
5 gcatggtgtctgggacctcttactctctcaacacagcagagtgagggtgaagatgctgaccttattactgcagcagtgaggattttaaacc
caccacagttcgggtctgggacaaagctggagctgaagaatggcgggtgctcgggcgggtggaactggagggaggagtgctca
ggctatctacagcagcttggggctgagctggggcctcagtgaaagtgtcctgcaggcttcgggtacacattaccaggt
acaatatgactgggtaagcagacacctagacaggcctggaatggattggagctatttatccaggaagtgtgatacttctcaatcag
aagttcaaggcgaagccacactgactgtgacaaaatcctcagcacagcctacatgcagctcagcagcctgacatctgaagactctcg
10 gttctatttctgcaagagtggtgactatagtaactcttactggtacttcgatgtctggggcacaggaccacgggtcacccgtctctctgatca
ggagcccaaatcttggacaaaactcacacatgtccaccgtccccagcacctgaactcctgggggaccgtcagctctctctctccccca
aaccgaaggacacccctcatgactcggacccttgaggtcacatgcgtgggtgggacgtgagccacgaagaccctgaggtcaagttc
aactgtgactgtggcggcggtggaggtgcataatgccaaagacaaagccggggaggagcagtaacaacgacgtaccgtgtggtgacg
gtcctcaccgtctgcacaggactgtgctgaatggaaggagatcaaatgcaaggctctccacaagaagccctccagcccccacgtgaa
15 aacaatctcgaagccaaaggcgaccccgagaaccacagggtgtacacctgccccatccgggatgagctgaccaagaacaggtc
agctgactgctcgtgcaaggctctatccagcgacatgcctgtgagtgaggagagcaatggcgacccggagaaacaactacaaga
ccagcgtcccgctgctgactccgacgctccttctctacagcaagctcaccgtggacaagagcaggtggcagcagggggaacgtct
tctcatgtccgtgatgcatgaggctctgcacaaccactacacgcagaaggacgtcctcctgctcctgggtaaatgatctaga

20 AA

2H7 scFv MTH (CCS) WTCH2CH3

mdfqvqifslisaviiarqivlsqspailsaspgkvmtcrasssvsynhwyqqkpgsspkpwiyapn
lasgvparsfgsgtsysltisrveadaatyccqwsfnpptfgagtkleldggsgggsgggsgaylqqsgaelvrpgasvk
msckasgyfttsynmhvkwktpqrqglewigaiypngdtsynqkfkgkatltvdksstmaymlsitsedsavfyfcarvvyysns
25 ywyfdvwtggttvssdqpksdkthtppspapellggpsvflfpkpkdtlmisrtpetvictvvdvshdepvkfnwyvdgv
evhnaktprceeqynstyrsvsvltvlhqdwlngkykckvsnkalpapietiskakgqprepqvlylppsrdelktngvslctlvk
gfypsdiavewesngqpennyktppvldsgsfllyskltvdksrwqqgnvfscsvmbhealhnhytqslslspgk

30 NT

HuIgAHIgA-T4-ORF

tgatgcacagttccctcaactccacctaccctatcctcctcaactccacctaccctatcctcctatgctgcaccccc
cgactgtactgcacgcagccgacctcgaggacctgctctagggttcagaagcgnatcctcagctgcacactgaccggcctgagagatgcc

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aa g c t t a t g a t t t t c a a g t g c a g a t t t c a g c t t c c t g c t a a c a g t g c t t c a g t c a t a a t g t c c a g a g g a g t g a c a t t
g t g c t c a c t a g t c t c c a a c a c c a t a g c t g c a t c t c a g g g a a g t g c a c c a t c a c c t g c g t g c c a g t c c a t g t a a g t a c a t g t a
c t g t a c c a g c a a g t c a g g c c t c c c t a a a c t c t g g a t t a t g a c a c a t c c a a g t g g c t t c g g a g t t c a a a t c g c t t c a g t g c a
g t g g t c t g g g a c c t t a t t c t c t g c a a t c a a c a c c a t g g a g a c t g a a g a t g c t g c c a t t a t t a c t g a g c a g t g g g a t a g t a c t c g c t
5 c a c g t t c g g g t c g g a c c a a g t g g a g a t c a a a c g g g t g g c g g t g g c t c g g g c g g t g g t g g g t c g g g t g g c g g c g g a t c a g g t g
c a g c t g a a g g a g g c a g g a c c t g c c t g t g c a a c g a c a c a g a c c c t g t c c c t a c a t g c a c t g t c t g t g t t c t t a a c c a g c a t g
g t g t a c a c t g a t t c a c a g c c t c c a g g a a g g t c t g g a a t g a t g g a a t a t a t a t a t g a t g a g g c a c a g a t t a a t c a g c a a t t a
a a t c c a g c a t g a g c a t c a g c a g g g a c a c c t c c a a g a g c c a a g t t t t c t a a a a t c a a c a g t c t g c a a a c t g a t a c a c a g c c a t g a t t a c
t g t g c a g a a t c c a t t t g a t t a c t g g g c c a a g g a g t c a t g t c a g t c t c c t g t a t c a g c a g t t c c t c a a c t c a c c t a c c a t c t c
10 c t c a a c t c c a c t a c c c a t c t c c c a t g t g c c a c c c c g a c t g t c a c t g c a c c a c c g g c c c t g a g a g a c c t g c t c t t a g g t t c a g a a
g c g a t c c t c a c g t g c a c a t g a c c g g c c t g a g a g a t g c c t c a g g t g c a c t t c a c c t t g g a c g c c c t c a a g t g g g a a g a g c g c t g t c a a
g g a c c a c c t g a c c g t g a c c t c t g t g g c t g a c a g c g t g c a g t g t c c a g t c c c g g c t g c c g a g c a t g a a c c a t g g g a a g a c c t c
a c t t g c a c t g t g c t c a c c c g a g t c c a a g a c c c g c t a a c c g c c a c c c t c t a a a t c c g g a a a c a c a t t c c g g c c g a g t g c c a c c t g
c t g c c g c c g c t c g g a g a g c t g c c c t g a a c g a g c t g t g a c g t g a c g t g a c g t g c c t g g c a c g t g g c t t a g c c c a a g g a t g t g c t g t
15 t c g t g c t g c a g g g t g c a c a g a g a g t g c t g c c c c g c g a g a a g t a c c t g a c t t g g g c a t c c c g a g g a g c c c a g c c a g g g c a c c a c c a c
c t t c g t g t a c a g c a t a c t g c g c t g c a g c c g a g g a c t g g a a g a a g g g g a c a c c t t c t c t g a t g t g g c c a c a g a g g c c c t g c
c g c t g c c t c a c a c a g a a g a n c a t c g a c c c g t t g g c g g g t a a a c c a c c a t g t c a a t g t g t c t g t g t a t g c g g a g g t g g a c g c g g
a t c c t c g a a c a c c t g c t c c a t c c t g g c c a t t a c c t a a t c t a g t a a a t g g a a t t t t g t a t a t g t c t g c t g a c t a c t g c t t g c c c a a
g a t c a g a g a g a g a a g g a g a a t g a g a g g a a a g t g t a c g c c c t g t a a a t c g a t a c

20

AA

ID8 scFv IgAH IgA-T4-CD80

m d f q v q i f s l l i s a s v i m s r g v d i v l t q s p t t i a a s p g e k v t i r c a s s v s y m y w y q q k s g a s p k l w i y d t s k l
a s g v p n r f s g s g t s y s l a i n t m e t e d a a t y c q q w s s t p l t f g s g t k l e i k r g g g s g g g s q v q l k e a g p l v q t p t q l s t
25 a s g v p n r f s g s g t s y s l a i n t m e t e d a a t y c q q w s s t p l t f g s g t k l e i k r g g g s g g g s q v q l k e a g p l v q t p t q l s t
c t v s g f s l t d g v h w i r q p p g k l e w m g i i y d g d t d y n s a i k s r l s i r d t s k s q v f l k i n s l q d t d a m y e a r i h f d y w g q g v m
v t v s s d q p v s p t p t p s p t p t p s p c h p r l s l h r p a l e d l l l g s e a i l t c t l g d a s g v t f w t p s s g k s a v q g p p d r l c g c y s v s s
v l p g c a e p w n h g k t f t c a a y p e s k t p l t a t i s g n t f i p e v h l p p p s e e l a n e l v t l e l a r g f s p k d v l r w l q g s g e l p r e k y l t w
a s r q e p s g t t t f a v i s l i r v a e d w k k g d t f s c m v g h e a l p l a f l q k t i d r l a g k p t h v n v s v m a e v d a d p s n n l p s w a i l i s v n
30 g i f v i c c t y c f a p r c r e r m e r l m s v r p v

NT

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human IgE Fc (CH2-CH3-CH4) ORF:

gtatcacgtctgctccagggaacttcacccgccaccgtgaagatcttiacgtcgtcctgcagccggcgccggcac
ttccccccaccatccagctctctgtgctcgtctctgggtacacccagggaactatcaacatcacctggctggaggccggcgaggtcatgg
acgtggactgtgtccaccgctctaccacgcagggaggtgagctggcctccacacaaagcgagctcacctcagccagaagcactgctgtg
5 tcagaccgcacctacacctgccaggtcacctatcaagggtcacacctttgaggacagcaacnaagagtgctgcaattccaacccgagagg
gtgagcgccctacctaagccggccagccggttcgacctgttaccgcaagtcgcccacgacacgtctgtctgggtggacctggcacc
agcaaggggaccgtgaacctgacctgggtccgggccaagtgggnaagcctgtgaaccactccaccagaanaaggagagaaagcagcgcaat
ggcacgttaaccgtcacgtcacccctgcccgtgggcaaccgagactggaatcagggggagacctaccagtgacgggtgaccaccccc
acctgccaggcgccctcatcggttcacgaccaagaccagcggcccgcgtgctgccccggaagcttatgctgtttgcgacgccggagtg
10 gccggggagccggggaacgcacccctgcctgctgacccaacttcatgctgaggaacatctcgtgtgacgtggctgcacaacgag
gtgcagctccggagcggccggcacagcacgacgcagccccgaagaccagggtccggctctctgcttcacggccctggaggtgna
ccaaggccgaatgggagcagaagaatgattcatctgcccgtgcagtcctatgagggcagcgagcccccacagaccgtccagcgagcgtg
gtctgtaatacccgtaaaagggaatcctcgaa

15

AA

human IgE Fc (CH2-CH3-CH4) ORF:

dhvcsrdtpptvkilqssedggghfpptqlclvsytpgtinitwledgqvmdvdlstastqegelastqseltl
sqkhlwlsdrtycqvttyqhtfedstkkcadnsnprgvsaylsrpspdlfrksptitclvddlpskgtnlntwsragkcpvnhstrkee
20 kqrngltlvtstlpvgtrdwiagetqqrviplphlpralmrstktsgpraapevyafatpewpgsrdrktlaclqnfmpedisvqwh
nevqlpdarhsttpkrktgsgffvfrlvttraewqkdeficravheaaspsqtvravsvnpgkadps

25

NT

1D8 scFv-human IgE Fc (CH2-CH3-CH4)-CD80

aagcttatggattttcaagtgcagattttcagctctctctaatactgcttcagtcataatgtccagaggagtcgacatt
gtgctcactcagctccacaacactagctgcatlccaggggagaaagtcaccatcacctgccgtgccagctccagtgtaagttaactgta
ctgtaccagcagaagtcaggcgccctccctaaactctgatttatgacacatccaagctggcttctggagttccaatlcgctcagtgcca
20 tgggtctgggacatcttattctctcgaatcaacacatggagactgaagatgctgccattattactctcagcagctggagtagtactccgct
cagcttcgggtgtgggaccaagctggagatcaaacgggggtggcggtggctcggggcggtggtgcggcgccggtacatcaggtg
cagctgaaggagcgaggacgtcgtggtgcaaccgacacagaccctgctccctacatgcaactgctctcgggttctcattaaacgcgagtg
gtgtacactgattcgacagccctcaggaaaggctctggaatggatgggaataatataattatgafggaggccagagattataatcagcaatta
aatccagactgagcagcagcagggaacctccaagagccaaggtttcttaaaaaatcaacagctctgcaactgafgacacagccatgtattac

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15

mdfqvqj f sflisavinsrgvdivlqspitiaaspgelvticirassvsvmywywqksgspklwyidskl
gtsyslain tmetedaatyycqwssplf gsgtkleikrgggsgggsgggsggvqlkeagpglvqptqlsl
wlrppgkclg lewmgiy ydggdynsaiksrlsrdtskqvfklnslqtdtdamycarihfdywgggvn
tpptvkilqssc dggghfpptjcllcvsgytpgtinitwledgqvmvdvlstastqceqelastqseltsqkhwsl
edstckca dsnprgsavysrpspdlfirkspitclvdlapskgtvnltwsraskpvnhrstkeekqmgltvt
tyqcrvth phlpralmrstktsgpraapevyafatpewpgsrkrlaclqnfmpedisvqwlhnevqlpar
tyqfslvletraeweqkdeficraveaaspsqtqrvavsvnpgkadpsklpswaitlisvngifvclctycfaprc
nv

25

5B9-IgA-H IgA-T4-CD80

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ggatcgtcacaggtgcagctgaagcagtcaggaacctggcctagtgcagtcctcacagagcctgtccatcacctgcagactctctgtttctc
 attaatctactatgctgtactactgggttcgccagctctccaggaaaagggtctggagtgctgggagtgatagtgagtggtggaatcacagact
 ataatgcagctttcatctccagactgagcatcaccaggagcattccaaagagccaaagttttcttaaaatgaacagctctgcaacctaatagacac
 agccattattactgtgcagaaaatgggggtgataactacccttattactatgctatggactactgggggtcaaggacctcagtcaccgtctcc
 5 tctgatagccaggtccctcaactccactacccatctccctcaactccactaccccatctccctcatgctgccacccccgactgtcactgc
 accgagccggcctcgaaggacctgctcttaggttcagaagcagctcactgcacactgacccgctgagagatgctcaggtgtacactt
 cactctggagccctcaagtgaggagagcgtgtlcaaggaccactgaccgtgacctctgtggctctacagcgtgtctcagtgctctgcc
 gggctgtgccgagccatgggaacctgggaagaccttcaactgctgctgctaccccgagtccaagacccccgtaaacgccaccctc
 aaaatccggaacacatctccggcccgagggtccactgctgccgccgccgctggagagagctggccctgaacgagctggtgagcgtgacg
 10 tggctggcactggtccttcagcccgaagatgtgctggttcgctgctgctcaggggtcacagagagctgccccgagaagtaactgacttgg
 gcatcccgagagagcccccaggggcaccaccacttctgctgtgaccagcactgctgctgagcagccgagagactgggaaggggg
 gacacctctctctgcatgggtgggcacagggccctgcccgtggccttcacacagaacacatcgaccgctggcggttaaacccaccat
 gtcaatgtgtctgttcatggcgaggagtgagccggatcttcgaacaacctgtccatctggcggttaaacccaccat
 attttgtgatagctgctgacgactactgctttgccccaagatgcagagagagagaggaaggaaagagagatgagaaggaaaggtgtacgcc
 15 ctgtataaatcgatgc

AA

5B9-IgAH IgA-T4-CD80

mrfsaqlllglvlwipgstadivmiquaafsnpvtlgtasiscsrsskllhsngitylywylqkpgqspqliiyqms
 20 nlasgvprdfsssgsgtdflrisrveadvgvyvcaqnlelplltfagtklelkrggsgggsgggsgggssqvlkqsgplqvssqsl
 sitctvsgfsltyavhwrvpyspgkglewlgviwsggtdynaafisrlsitkddsksqvfkmmnslqpdntaiyyccarnagdnyppy
 amdwygggtsvtvssdqpvpstppstppstppscchprlsihrapledlllgseailctgltdrdsagvftvtpssgksavagpp
 drdlegcysvssvlpgeapwnhgtctfttaaypeskptlatlsksntfrpevhlpppseelaenlvtctclargfspkdvrvrlqg
 sqelprekyltwasrqepsqgtttfavtsilrvaedwkgdgtfscmvghaelplafitqktidrlagkphvrvsvmaevdadpsnnl
 25 lpswaitlisvngifviclctyefaprcrrrrrrrrrrresrvp

NT

5B9-scFv-human IgE Fc (CH2-CH3-CH4)-CD80

aagcttgcgcccatgaggttctctgctcagcttctggggctgctgtgctctgagccctggatccactgcagatattgt
 30 gatgacgcagcgtcattctccaatccagctactcttgaacatcagcttccatctcctgcaggtctagtaagaactcctcatagtaagtc
 atcactattttgatttgctatctcagaagccaggccagctcctcagctcctgattatcagatgtccaaactgtgccagaggtccagacag
 gttcagtagcagctgtgacgaagcatttcacactgagaaatcagcagagtgaggctgagagtgatgggtttattactgtgctcaaaatct

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180

PCT/US2003/024918

[illegible]

181

PCT/US2003/024918

aagcttatggatttcaagtcagatttcaagcttctgctaatacagtgcttcagtcataatgtccagaggagtcgacatt

25

2e12-scFv-human IgE Fc (CH2-CH3-CH4)-CD80

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isvqwlhnevqlpdarhstprkrtksggffvfrlevtraewqkdeficravheaaspsqtvqravsvnpgkadpsklpswaitlsv
ngifvicltyclaprcrermerlrrsvrvp

NT

5 500A2 scFv

atgttgatatactcagctcctgggctttactctcttggttcagcctccagaagtgacatagtgtgactcagactc
cagccactctgtctctaattcctggagaaagagtcacaatgacctgtaagaccagtcagaatattggcacaaatcttactcggatcaccaaa
aaccaaaggaggctccaaaggctctc atcaagtatgcttcgagtcattcctgggatccctccagattcagtggcagtggttcggaaca
gattcactctcagcatcaataaccttgagcctgatgatcggaaatttattactgtcaacaaagtagaagctggcctgtcacgttcggctcgtg
10 gcaccaagctggagataaaacggcggtggcgtgctcggcgagggtgggtcgggtggcggcggtatctcaggtcaagctgcagcagtg
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cacactgactgtagataaaatctcta gcacagcctacatggaactcagcagcctgacatctgaggattctgccatctattactgtgcaagaag
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15 AA

500A2 scFv

mlytsqllgllfwisarsdivltqtpatlslipegervtmctksqniqtilhwyhqkpkpeapralikyassqisipip
srfsgsgsetdfinnlepdidgiyycqqsrswpvtfgptkcleikrsgggsgggsgggsgvklqsgselgkpgasvklsccts
20 gyiftdhyiswvkqkpgeslqwignvvyggnggtsynqkfgkattlvdkisstaymelsltsedsaiyyccarppvatghamdywg
qgiqvtvssd

5' oligo:

25 Name : hIgAbel5
Sequence : GTTGTGTGATCAGCCAGTTCCTCAACTCCACCTACC

3' oligo:

Name : IgA3BB
30 GTTGTGTTTCGAAGGATCCCGCTCCACCTCCGCCATGACAACAGA

5' oligo:

Name : IgGWT3

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GT'TGTTTTCGAAGGATCCGCTTTACCCGGAGACAGGGAGAGGCTCTT

3' oligo:

Name : hIgGWT5

5

GTTGTTAGATCTGGAGCCCAAATCTTGTGACAAAACACACATG

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5' oligo:

Name : FADD5

Sequence

5 GTTGTGGATCCTTCGAACCCGTTCTGGTGTCTGCTGCACTCGGTGTCTG

3' oligo:

Name : FADD3

Sequence

10 GTTGTATCGATCTCGA GTTATCAGGACGCTTCGGAGGTAGATGCGTC

FADD-CSSCFV:

Gtggatctctgaacccgttctctggtgctgctgactcgggtgctgctcagccctgctgagcagcgagctgaccgagct
caagttctatgacctggcgcgctgggccaagcgaagctggagcgcgtgagagcggcctagacctcttctcatgctgctggagcaga
15 acgacctggagcccgccacaccgagctcctgctgagcagctgctcgcctccctgcgggcgccacgacctgctgcgcgctgacgactt
cgagcgggggggcgccggcgccggcgccgctgggggaagaaacctgtgtgacgacatttaacgtcatatgtgataatgtgggaaagat
tggagaaggctgctcgtcagctcaaaagtctcagacaccagatcgacagcagcagacagataccccgcaacctgacagagcgtgt
gcgggagtcactgagaatctggaagaacacagagaaggagaacgcgaacagtgccacactggtgggggctctcaggtcctgccagatg
aacctggtgctgacctggtacaagagggtcagcagcccgtagcttcagaacaggagtggggccatgtccccgatgctatggaactc
20 agacgcatctacctcgaagcgtcctgataactcgagatcgatacaaac

Peptide sequence:

vdpsnpflvllhsvssslssstcltkfclgrvgrkrklervqsgldlfsmlleqndlepghltelllaslrhdllrrv
ddfeagaaagaapgeedlcaafnvcnvgkdwrrlarqlkvsdtkidsiedryprnltervreslrwknktenatvahlgalscq
25 mnlvadlvqevqqardlqnrgamspmswnsdastseas

Name : HCD28tm5B

GTTGTGGATCCTCCCTTTTGGGTGTCTGGTGGTGGTTGGTGTCTGCGCIT

30 GCTATAGCTTG

Name : HCD28tm3S

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GTTGTTTCGAACCCAGAAAATAATAAAGGCCACTGTTACTAGCAAGCT
ATAGCAAGCCAG

HCD28tm5'
5 GTTGTGGATCCTCCCTTTTGGGTGCTGGTGGT
HCD28tm3'
GTTGTTTCGAACCCAGAAAATAATAAAGGCCAC

10 HCD80tm5'
GTTGTGGATCCTCCTGCTCCCATCCTGG
HCD80tm3'
GTTGTTTCGAACGGCAAAGCAGTAGGTCAGGC

15 Name : MFADD5BB
Sequence :
GTTGTGGAATCCTTCGAACCCATTCTGTGTGCTGCTGCAC'TCGCTG

20 Name : MFADD3XC
Sequence :
GTTGTTATCGATCTCGAGTCAGGGTGT'TTCTGAGGAAGACAC

25 Murine FADD Nucleotide sequence (full length, but without flanking -Ig or
transmembrane sequences):

Gtggatccttcgaacatggaccattcctggtgctgctgcactcgtgtccggcagcctgtcgggcaacgatcgtat
ggagctcaagtctctgtgccgcgagcgcgtgagcaaacgaaagctggagcgcgtgcagagtggtccctggacctgttcacgggtgctgctg
agcagaacgacctggagcgcggggcacaccgggctgctgcgcgagttgctggcctcgtgcgcgcgacacgatctactgcagcgcctgga
cgacttcgagcggggacgcgcgaccgtcgcgccccgggggagcgagatcgcaggtggcattgacaltgtgtgtgacaatgtgggg
30 agagactggaaaagactgcccgcgagctgaaggtgtctgaggccaagatggatggattgaggagaagtacccccgaagtctgagtg
agcgggttaaggagagcttgaaggtctgggaagaatgctgaagaaagacgcctcgggtggccgactggtcaaggcgtcgcggaact
gcaggctgaatctgtggctgacctgtgtggaagaagcccaaggaalctgtgagcaagatgagaatalgtccccgactaagggaaltcaa
ctgtgtcttcctcagaacaacccctgactcgagatcgat

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Murine FADD

vdpsnmdpfllvllhsisgslsgndlmelkflcrervskrklerlvqsgldlftvlleqndlerghtglirellaslrhddll
qrlldfeagtataappgeadlqvafdivcdnvgrdwkrlarelkvseakmdgieekyprslservreslkvwknackknasvaglvk
5 alrtcrlnlvadlvecaqesvksenmspylrdstvsstetp

Name : MCASP3-5

10 Sequence :

GTTGTGGATCCTTCGAACATGGAGAACAACAAAACCTCAGTGGATTCA

Name : MCASP3-3

Sequence :

GTTGTTATCGATCTCGAGCTAGTGATAAAAGTACAGTTCTTTCGT

15

Name : mcasp8-5

Sequence :

20 GTTGTTTCGAACATGGATTTCAGAGTTGTCTTTATGCTATTGCTG

Name : mcasp8-3

Sequence :

GTTGTTATCGATCTCGAGTCATTAGGGAGGGAAGAAGAGCTTCTTCCG

25

Name : hcasp3-5

Sequence :

GTTGTGGATCCTTCGAACATGGAGAACAACGAAACTCAGTGGAT

Name : hcasp3-3

30 Sequence :

GTTGTTATCGATCTCGAGTTAGTGATAAAATAGAGTTCTTTTGTGAG

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Sequence

GTTGTGGATCCTTCGAACATGGACTTCAGCAGAAATCTTTATGAT

Name : hcasps8-3

5

Sequence

GTTGTTATCGATGCATGCTCAATCAGAAGGGAAGACAAGTTTTTTTCT

HuIgGMHWC

10 gtt gtt gat cag gag ccc aaa tct tct gac aaa act cac aca tct cca ccg tcc cca gca cct gaa
ctc ctg ggt g ga cgg tca gtc ttc c

NT

15

2H7-human IgE (CH2-CH3-CH4)

aagctgcgcccatggaatttcaagtcgaaattttagcctctcgtacatcagtgcttcagtcataattgccagaggaca
aatgtgtctccacagtcctcagcaalctgtctgcaltctccaggaggagaaagtcacaaagacttcgaggccagctcaagtgtaagttalac
gcatctggtaccacagagaaagccaggatctctccccaacacctgtgatttatgcccaatcacaacctgtctctgaggtccctgtctgcttcagtg
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20 caccacagctctgggtctgggaccacaagctggagctgaagagtggtgggtggtctggggcgtggtggtgatctggagagagtggtggagctctca
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25 ctgtctcaggggacttcaaccgccccacacgtgaagattttacagtcgtctctgcgacggcggtgggacattccccgccacatcagctctct
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accacgcaggaggggtgagctgctccacacaagcggagctcacctccagccagaagcactggctgtcagaccgcactacacactgccc
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30 acctgtgtcccgccgacgtgggaagcctgtgaacacactccaccagaaggaggaagcagcgcgaatggcacttgaacctgcactccca
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ggacccctgcctgcctgtatcaagaacttactgtctgaugaaatctgctgactctgtctcacaacaggtgtcaactcccgacgccctgc

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cacagcacgacgacgccccgaagaccaaggctccgctctctcgtcttcagccgctggagggtaccagggccgaantgggagcaga
aagatgagttcatctgccgtgcatgcatgagcagcgagccctcacagaccgtccagcgagcggtgtctgtaaatcccggtaaatgata
atctaga

5

AA

2H7 scFv IgE (CH2-CH3-CH4)

mdfqvqifsfllisaviiargqivlsqspailsaspgekvtmtrassvsmhwyqqkpgsspkpwiypasn
lasgvparfsgsgsgtsylstrveaedaatyycqqwsmfpptfagtklelkgggsgggsgggssqaylqqsgaelvrpgasvk
msckasgytfsynmhvkvtpqrqglewigaiypngdtsynqkfkkgkatltvdkssstaymqlsslsedsavfyfcarvvyysns
10 ywyfdvwtggttvtvshvcsrdlfpptvkilqsscdggghfpptqllclvsgytpgtinitwiedgqvmvdvlstasttqgelastqs
eltlsqkhwlsdrtyctvtyqghftedstkkcadsnprgsaylsrpsfdllfirkspitclvdlapskgtvnltsrasgkpvnhstr
keekqrmgtltvtlpgvtrdwiegetyqcrvthphlpralmrsttktsgrapapevyafatpewpgrsdrklaciqlnfmpedisq
wlhnevqlpdrhsttpqrktksggfvsrlvtraewekdeficra vheaaspsqlqvrasvnpkg

15

NT

2H7 scFv MH (SSS) MCH2.WTCH3

aagcttgcgcgcaatggattttcaagtcagcagattttcagcttccgtctaatacagtccttcagcagataattgcagaggaca
20 aattgttctctccagctctccagcaatcctgtctgcatctccagggaagaaggtcaaatgacttgcaggcgcagctcaagtgtaaagtacat
gcactgtgtaccagcagaagccaggatctccccaaacctggatttatgccccatccaacotgcttctgagtcctcgtcgttcagtg
gcactgggtctgtggacacctctactctcacatcagcagagtggaaggctgaagatgctgccactattactgccagcagtggaagttaaacc
caccacagctcgggtgctggaccaaagctggagctgaaagatggcgggtgctcgggcggfctggfagctgagaggaagggtggagctctca
ggcttatctacagcagcttggggctgagctgtgtgagccctggggcctcagtgaaagatgtctctcaaggcttctgctacacattaccaggt
25 acaatgatcactgggtaaagcagacacctagacaggccctggaatggattggaagctatttatcaggaaatggtgatacttctcacaatcag
aagttcaaggggcaaggccacactgactgtagacaatccctccagcagcgtactatcagcgtcagcagcgtgacatctgaagactctcgcg
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ggagcccaaatctctgacaaaactcacacatccccaccgtccccagaccctgaactctctgggggagatcgtcagcttctcttcccccaaa
aaccacaggaacaccctcatgactcccgaccocctgaggtcacaatgcgtgtgtgtggacgtgagccaggaagaccctgaggtcaagtctc
30 aactgtgacgtgtgagcggcgtggaagggtcataatgccaaagacaaagccgcggggaggagcaglacaaacagcacgtaccgtgtgtcagc
gtctcaccgtctgcacacgaagctggtgaatggcaaggagtacaagtgcaaggtctccaaagagccctccagcccccacatcgagaa
aacaatctccaaagccaaagggaagcccccgaagaccacaggtgtaacccctgccccatccgggagatgagctgaccaagaacacaggtc
agcgtcagctgctggtgcaaaaggcttctatccacgcagacatgccgtgagtgaggagagcaatgggacggcggaagaacatacaga

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AA
2H7 scFv MH (SSS) MCH2WTC^{H3}
mdfqyqifsfllisaviiargqivlsqspailsaspgekvtmtcrassvsymhwyqkqgsspkpwiyapsn
lasgvparfsgsgsgtsysltirveadaatyccyqwfnpptfigagtkleldggsgggsgsgsgsqaylqqsgaelvrgpagsvk
msckasgytftsynmhvkkptprqglewigaiypngndtsynqkfkgkatltvdksstaymqlslltsedsavycfarvvyysns
ywyfydvwgtgttvtsddqepkssdkthtspspapellggssvflfppkpkdltimirstpvtcvvvdshdedpevkfinwyvdgv
evhnaktkpreegynstyrvtvltvhlqdwlingekykckvsnkalpapietkiskakgapprepvytlpssrdeltknqsvtlvkv
gfy-psdiavewesngapennyyktppvlvdsdgsfllvykcltvdksrwaqgnvfscsvmhcalhnhvtakslslspek

5B9 scFv MTHWTCH2CH3

[illegible]

AA

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mrfsaqlglglwlpwpgstadvmtqaafsnvptlgtssasiscersksllhngitylywylqkpgqspqlliyqms
nlasgvpdrfsssgsgdftlfrsiveaedvgyycaqnlclpftfagtkcllkrgeegsgggsgggsgsgvqlksgpqlvassql
sictvcgslfityavhwvrqspgkglewlgviwsggitynaafisrlsitkddsksgvfikmnsqpnidaiyycarnggdnpypy
amdywggqstvssdqepkssdkthtspspapellggpsvflfppkpkdtlmirsktgpevcvrvvdshedpevkfinwydvdge
vhnaktwreepqynstyrsvsvltvdlwqhngkecykcsnawpaktiektsaktpgpcpvcvtytppsrddlqnqslvtclvkgf
ypsvdiavecsnagennnyktpptvdsdglgfyfsvklvksrwaqgnfiscvsmhaelhhnytkqslsslspek

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for the purpose of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the present invention is not limited except as by the appended claims.

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CLAIMS

1. A binding domain-immunoglobulin fusion protein, comprising:
 - 5 (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide is selected from the group consisting of (i) a wild-type human IgG1 immunoglobulin hinge region polypeptide, (ii) a mutated human IgG1 immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human
10 IgG1 immunoglobulin hinge region polypeptide contains two cysteine residues and wherein a first cysteine of the wild-type hinge region is not mutated, (iii) a mutated human IgG1 immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 immunoglobulin hinge region polypeptide contains no more than one cysteine residue, and
15 (iv) a mutated human IgG1 immunoglobulin hinge region polypeptide that is derived from a wild-type immunoglobulin hinge region polypeptide having three or more cysteine residues, wherein said mutated human IgG1 immunoglobulin hinge region polypeptide contains no cysteine residues;
 - (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to
20 the hinge region polypeptide; and
 - (c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide,
wherein:
 - (1) the binding domain-immunoglobulin fusion protein is capable of at least
25 one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and
 - (2) the binding domain polypeptide is capable of specifically binding to an antigen.
- 30 2. An isolated binding domain-immunoglobulin fusion protein comprising: a) a binding domain polypeptide capable of specifically binding to an antigen; b) an immunoglobulin hinge region polypeptide fused to said binding domain polypeptide, said immunoglobulin hinge region polypeptide comprising first, second, and third cysteine residues, where said first cysteine residue

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is N-terminal to said second cysteine and said second cysteine is N-terminal to said third cysteine, wherein said first cysteine residue is not mutated and one or both of said second and third cysteine residues is substituted or deleted; and c) an N-terminally truncated immunoglobulin heavy chain constant region polypeptide fused to said immunoglobulin hinge
5 region polypeptide, wherein the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation.

3. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding
10 domain polypeptide comprises at least one immunoglobulin variable region polypeptide that is selected from the group consisting of an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide.

4. A binding domain-immunoglobulin fusion protein according to claim 3 which comprises
15 an immunoglobulin heavy chain variable region polypeptide, wherein said heavy chain variable region polypeptide is a human immunoglobulin heavy chain variable region polypeptide comprising a mutation at an amino acid at a location corresponding to amino acid position 11 in the first framework region of the heavy chain variable region.

20 5. A binding domain-immunoglobulin fusion protein according to claim 3 which comprises a polypeptide having a sequence selected from the group consisting of SEQ ID NO: __ and SEQ ID NO: __.

6. The binding domain -immunoglobulin fusion protein of claim 3 wherein the
25 immunoglobulin variable region polypeptide is derived from a human immunoglobulin.

7. The binding domain -immunoglobulin fusion protein of claim 3 wherein the
immunoglobulin variable region polypeptide comprises a humanized immunoglobulin polypeptide sequence.

30

8. The binding domain-immunoglobulin fusion protein of claim 3 wherein the immunoglobulin variable region polypeptide is derived from a murine immunoglobulin.

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9. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises:
 - (a) at least one immunoglobulin light chain variable region polypeptide;
 - (b) at least one immunoglobulin heavy chain variable region polypeptide; and
 - 5 (c) at least one linker polypeptide that is fused to the polypeptide of (a) and to the polypeptide of (b).
10. The binding domain-immunoglobulin fusion protein of claim 9 wherein the immunoglobulin light chain variable region and heavy chain variable region polypeptides are derived from human immunoglobulins.
11. The binding domain-immunoglobulin fusion protein of claim 9 wherein the linker polypeptide comprises at least one polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO: ____].
12. The binding domain-immunoglobulin fusion protein of claim 9 wherein the linker polypeptide comprises at least three repeats of a polypeptide having as an amino acid sequence Gly-Gly-Gly-Gly-Ser [SEQ ID NO: ____].
13. A binding domain-immunoglobulin fusion protein according to claim 9 wherein the linker comprises a glycosylation site.
14. The fusion protein of claim 13 wherein the glycosylation site is selected from the group consisting of an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site and a phosphoglycation site.
15. The binding domain-immunoglobulin fusion protein of claim 1 wherein at least one of the immunoglobulin heavy chain CH2 constant region polypeptide and the immunoglobulin heavy chain CH3 constant region polypeptide is derived from a human immunoglobulin heavy chain.

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16. The binding domain-immunoglobulin fusion protein of claim 1 wherein the immunoglobulin heavy chain constant region CH2 and CH3 polypeptides are of an isotype selected from the group consisting of human IgG and human IgA.

5 17. The binding domain-immunoglobulin fusion protein of claim 1, 2 or 73 wherein the antigen is selected from the group consisting of CD19, CD20, CD22, CD37, CD40, L6, CD2, CD28, CD30, CD40, CD50 (ICAM3), CD54 (ICAM1), CD80, CD86, B7-H1, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, CD19, CD3, CD4, CD25, CD8, CD11b, CD14, CD25, CD56 and CD69.

10

18. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises a CD154 extracellular domain.

15 19. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises a CD154 extracellular domain and at least one immunoglobulin variable region polypeptide.

20. The binding domain-immunoglobulin fusion protein of claim 1 or 2 wherein the binding domain polypeptide comprises a CTLA-4 extracellular domain.

20

21. The binding domain-immunoglobulin fusion protein of claim 2 or 20 wherein at least one of the immunoglobulin heavy chain constant region polypeptides selected from the group consisting of a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgG1 constant region polypeptide.

25

22. The binding domain-immunoglobulin fusion protein of claim 20 wherein at least one of the immunoglobulin heavy chain constant region polypeptides selected from the group consisting of a CH2 constant region polypeptide and a CH3 constant region polypeptide is a human IgA constant region polypeptide.

30

23. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide;

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(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide; and

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide,

5 wherein:

(1) the binding domain polypeptide comprises a CTLA-4 extracellular domain that is capable of specifically binding to at least one CTLA-4 ligand selected from the group consisting of CD80 and CD86,

(2) the immunoglobulin hinge region polypeptide comprises a polypeptide
10 that is selected from the group consisting of a human IgA hinge region polypeptide and a human IgG1 hinge region polypeptide,

(3) the immunoglobulin heavy chain CH2 constant region polypeptide comprises a polypeptide that is selected from the group consisting of a human IgA heavy chain CH2 constant region polypeptide and a human IgG1 heavy chain CH2 constant region
15 polypeptide,

(4) the immunoglobulin heavy chain CH3 constant region polypeptide comprises a polypeptide that is selected from the group consisting of a human IgA heavy chain CH3 constant region polypeptide and a human IgG1 heavy chain CH3 constant region
polypeptide, and

(5) the binding domain-immunoglobulin fusion protein is capable of at least
20 one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation.

24. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region
25 polypeptide, wherein said hinge region polypeptide comprises a human IgE hinge region polypeptide;

(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a human
30 IgE CH2 constant region polypeptide; and

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a human IgE CH3 constant region polypeptide

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wherein:

- (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and induction of an allergic response mechanism, and
 - 5 (2) the binding domain polypeptide is capable of specifically binding to an antigen.
25. A binding domain-immunoglobulin fusion protein according to claim 24 that comprises a human IgE CH4 constant region polypeptide.
- 10 26. The binding domain-immunoglobulin fusion protein of claim 24 wherein the antigen is a tumor antigen.
27. A binding domain-immunoglobulin fusion protein, comprising:
- 15 (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of specifically binding to at least one antigen that is present on an immune effector cell and wherein the hinge region polypeptide comprises a polypeptide selected from the group consisting of a human IgA hinge region polypeptide, a human IgG hinge region polypeptide, and a human IgE hinge region
- 20 polypeptide;
- (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH2 constant region polypeptide, a human IgG CH2 constant region polypeptide, and a human IgE CH2 constant
- 25 region polypeptide;
- (c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH3 constant region polypeptide, a human IgG CH3 constant region polypeptide, and a human IgE CH3 constant
- 30 region polypeptide; and
- (d) a plasma membrane anchor domain polypeptide.

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28. The binding domain-immunoglobulin fusion protein of claim 27 wherein the membrane anchor domain polypeptide comprises a transmembrane domain polypeptide.
29. The binding domain-immunoglobulin fusion protein of claim 27 wherein the membrane anchor domain polypeptide comprises a transmembrane domain polypeptide and a cytoplasmic tail polypeptide.
30. The binding domain-immunoglobulin fusion protein of claim 29 wherein the cytoplasmic tail polypeptide comprises an apoptosis signaling polypeptide sequence.
31. The binding domain-immunoglobulin fusion protein of claim 30 wherein the apoptosis signaling polypeptide sequence is derived from a receptor death domain polypeptide.
32. The binding domain-immunoglobulin fusion protein of claim 31 wherein the death domain polypeptide comprises a polypeptide selected from the group consisting of an ITIM domain, an ITAM domain, FADD, TRADD, RAIDD, CD95 (FAS/ Apo-1), TNFR1 and DR5.
33. The binding domain-immunoglobulin fusion protein of claim 30 wherein the apoptosis signaling polypeptide sequence comprises a polypeptide sequence derived from a caspase polypeptide that is selected from the group consisting of caspase-3 and caspase-8.
34. The binding domain-immunoglobulin fusion protein of claim 27 wherein the plasma membrane anchor domain polypeptide comprises a glycosyl-phosphatidylinositol-linkage polypeptide sequence.
35. The binding domain-immunoglobulin fusion protein of claim 27 wherein the antigen that is present on an immune effector cell is selected from the group consisting of CD2, CD28, CD30, CD40, CD50 (ICAM3), CD54 (ICAM1), CD80, CD86, B7-H1, CD134 (OX40), CD137 (41BB), CD152 (CTLA-4), CD153 (CD30 ligand), CD154 (CD40 ligand), ICOS, CD19, CD20, CD22, CD37, L6, CD3, CD4, CD25, CD8, CD11b, CD14, CD25, CD56 and CD69.
36. The binding domain-immunoglobulin fusion protein of claim 27 wherein the human IgG is human IgG1.

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37. A binding domain-immunoglobulin fusion protein, comprising:
 - (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of specifically binding to at least one antigen that is present on a cancer cell surface and wherein the hinge region polypeptide comprises a polypeptide selected from the group consisting of a human IgA hinge region polypeptide, a human IgG hinge region polypeptide, and a human IgE hinge region polypeptide;
 - (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH2 constant region polypeptide, a human IgG CH2 constant region polypeptide, and a human IgE CH2 constant region polypeptide;
 - (c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH3 constant region polypeptide, a human IgG CH3 constant region polypeptide, and a human IgE CH3 constant region polypeptide; and
 - (d) a plasma membrane anchor domain polypeptide.
38. The binding domain-immunoglobulin fusion protein of claim 37 wherein the membrane anchor domain polypeptide comprises a transmembrane domain polypeptide.
39. The binding domain-immunoglobulin fusion protein of claim 37 wherein the membrane anchor domain polypeptide comprises a transmembrane domain polypeptide and a cytoplasmic tail polypeptide.
40. The binding domain-immunoglobulin fusion protein of claim 37 wherein the membrane anchor domain polypeptide comprises a glycosyl-phosphatidylinositol-linkage polypeptide sequence.
41. The binding domain-immunoglobulin fusion protein of claim 37 wherein the human IgG is human IgG1.

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42. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein said hinge region polypeptide comprises a wild-type human IgA hinge region polypeptide;

5 (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a human IgA CH2 constant region polypeptide; and

(c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a
10 polypeptide selected from the group consisting of (i) a wild-type human IgA CH3 constant region polypeptide and (ii) a mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain,

wherein:

(1) the binding domain-immunoglobulin fusion protein is capable of at least
15 one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and

(2) the binding domain polypeptide is capable of specifically binding to an antigen.

20 43. The binding domain-immunoglobulin fusion protein of claim 42 wherein the mutated human IgA CH3 constant region polypeptide that is incapable of associating with a J chain is selected from the group consisting of (i) a polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:___ and (ii) a polypeptide comprising an amino acid sequence as set forth in
25 SEQ ID NO:___.

44. A binding domain-immunoglobulin fusion protein, comprising:

(a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide;

(b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to
30 the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a llama CH2 constant region polypeptide that is selected from the group consisting of a llama IgG1 CH2 constant region polypeptide, a llama IgG2 CH2 constant region polypeptide and a llama IgG3 CH2 constant region polypeptide; and

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- (c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a llama CH3 constant region polypeptide that is selected from the group consisting of a llama IgG1 CH3 constant region polypeptide, a llama IgG2 CH3 constant region polypeptide and a llama IgG3 CH3 constant region polypeptide

wherein:

- (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and induction fixation of complement, and
- (2) the binding domain polypeptide is capable of specifically binding to an antigen.

45. A binding domain-immunoglobulin fusion protein according to claim 44 wherein the immunoglobulin hinge region polypeptide, the llama CH2 constant region polypeptide and the llama CH3 constant region polypeptide comprise sequences derived from a llama IgG1 polypeptide and wherein the fusion protein does not include a llama IgG1 CH1 domain.

46. A binding domain-immunoglobulin fusion protein according to any one of claims 1, 2, 23, 24, 27, 37, 42 or 44 wherein the hinge region polypeptide is mutated to contain a glycosylation site.

47. The fusion protein of claim 46 wherein the glycosylation site is selected from the group consisting of an asparagine-linked glycosylation site, an O-linked glycosylation site, a C-mannosylation site, a glypiation site and a phosphoglycation site.

48. A binding domain-immunoglobulin fusion protein according to any one of claims 1, 2, 23, 24, 27, 37, 42 or 44 wherein the binding domain polypeptide comprises two or more binding domain polypeptide sequences wherein each of said binding domain polypeptide sequences is capable of specifically binding to an antigen.

49. A binding domain-immunoglobulin fusion protein, comprising:

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- (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein the hinge region polypeptide comprises an alternative hinge region polypeptide sequence;
- (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide; and
- (c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide,
- wherein:
- (1) the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation, and
- (2) the binding domain polypeptide is capable of specifically binding to an antigen.
50. A binding domain-immunoglobulin fusion protein, comprising:
- (a) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, wherein the binding domain polypeptide is capable of specifically binding to at least one antigen that is present on a cancer cell surface and wherein the hinge region polypeptide comprises an alternative hinge region polypeptide sequence;
- (b) an immunoglobulin heavy chain CH2 constant region polypeptide that is fused to the hinge region polypeptide, wherein said CH2 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH2 constant region polypeptide, a human IgG CH2 constant region polypeptide, and a human IgE CH2 constant region polypeptide;
- (c) an immunoglobulin heavy chain CH3 constant region polypeptide that is fused to the CH2 constant region polypeptide, wherein said CH3 constant region polypeptide comprises a polypeptide selected from the group consisting of a human IgA CH3 constant region polypeptide, a human IgG CH3 constant region polypeptide, and a human IgE CH3 constant region polypeptide; and
- (d) a plasma membrane anchor domain polypeptide.

51. A binding domain-immunoglobulin fusion protein according to either claim 49 or claim 50 wherein the alternative hinge region polypeptide sequence comprises a polypeptide sequence

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of at least ten continuous amino acids that are present in a sequence selected from the group consisting of SEQ ID NOS: __-__.

52. An isolated polynucleotide encoding a binding domain-immunoglobulin fusion protein
5 according to any one of claims 1, 2, 23, 24, 27, 37, 42, 44, 49 and 50.

53. A recombinant expression construct comprising a polynucleotide according to claim 52 that is operably linked to a promoter.

10 54. A host cell transformed or transfected with a recombinant expression construct according to claim 53.

55. A method of producing a binding domain-immunoglobulin fusion protein, comprising the steps of:

(a) culturing a host cell according to claim 54 under conditions that permit
15 expression of the binding domain-immunoglobulin fusion protein; and

(b) isolating the binding domain-immunoglobulin fusion protein from the host cell culture.

56. A pharmaceutical composition comprising a binding domain-immunoglobulin fusion
20 protein according to any one of claims 1, 2, 23, 24, 42, 44, 49 or 50 in combination with a physiologically acceptable carrier.

57. A pharmaceutical composition comprising an isolated polynucleotide encoding a binding
25 domain-immunoglobulin fusion protein according to any one of claims 1, 2, 23, 24, 27, 37, 42, 44, 49 and 50 in combination with a physiologically acceptable carrier.

58. The use of a compound according to claim 1 in the preparation of a medicament for the
treatment of a subject having or suspected of having a malignant condition or a B-cell disorder,
comprising administering to a patient a therapeutically effective amount of a pharmaceutical
30 composition selected from the group consisting of the pharmaceutical composition of claim 56 and the pharmaceutical composition of claim 57.

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59. The use of claim 58 wherein the malignant condition or B-cell disorder is selected from the group consisting of a B-cell lymphoma and a disease characterized by autoantibody production.
- 5 60. The use of claim 58 wherein the malignant condition or B-cell disorder is selected from the group consisting of rheumatoid arthritis, myasthenia gravis, Grave's disease, type I diabetes mellitus, multiple sclerosis and an autoimmune disease.
61. The use of claim 58 wherein the malignant condition is selected from the group
10 consisting of melanoma, carcinoma and sarcoma.
62. A binding domain-immunoglobulin fusion protein according to claim 2 wherein said second cysteine residue is substituted or deleted and said third cysteine residue is not substituted or deleted.
- 15 63. A binding domain-immunoglobulin fusion protein according to claim 62 wherein said second cysteine residue is substituted and not deleted.
64. A binding domain-immunoglobulin fusion protein according to claim 63 wherein said
20 second cysteine is replaced with serine.
65. A binding domain-immunoglobulin fusion protein according to claim 2 wherein said third cysteine residue is substituted or deleted and said second cysteine residue is not substituted or deleted.
- 25 66. A binding domain-immunoglobulin fusion protein according to claim 65 wherein said third cysteine residue is substituted and not deleted.
67. A binding domain-immunoglobulin fusion protein according to claim 66 wherein said
30 third cysteine is replaced with serine.
68. A binding domain-immunoglobulin fusion protein according to claim 2 wherein said second cysteine and said third cysteine residues are both substituted or deleted.

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69. A binding domain-immunoglobulin fusion protein according to claim 68 wherein said second cysteine and said third cysteine are substituted.
- 5 70. A binding domain-immunoglobulin fusion protein according to claim 68 wherein said second cysteine and said third cysteine are replaced with serine.
71. A binding domain-immunoglobulin fusion protein according to any one of claims 2, 62, 63, 64, 65, 66, 67, 68, 69, or 70 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide comprises an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.
- 10 72. A binding domain-immunoglobulin fusion protein according to any one of claims 2, 62, 63, 64, 65, 66, 67, 68, 69, or 70 wherein said N-terminally truncated immunoglobulin heavy chain constant region polypeptide consist essentially of an IgG CH2 constant region polypeptide attached to an immunoglobulin heavy chain IgG CH3 constant region polypeptide.
- 15 73. An isolated binding domain-immunoglobulin fusion protein comprising: a) a binding domain polypeptide capable of specifically binding to an antigen, said binding domain polypeptide comprising an immunoglobulin light chain variable region polypeptide and an immunoglobulin heavy chain variable region polypeptide, wherein the leucine at position 11 in the first framework region of said heavy chain variable region polypeptide is substituted or deleted; b) an immunoglobulin hinge region polypeptide fused to said binding domain polypeptide; and c) an N-terminally truncated immunoglobulin heavy chain constant region polypeptide fused to said immunoglobulin hinge region polypeptide, wherein the binding domain-immunoglobulin fusion protein is capable of at least one immunological activity selected from the group consisting of antibody dependent cell-mediated cytotoxicity and complement fixation.
- 20
- 25

30

FIG. 1A

2H7scFv-Ig cDNA and predicted amino acid sequence:

```

HindIII      NcoI      2H7 VL Leader Peptide→
-----
1  AAGCTTGCCG CC  ATGGATT TCAAGTCAG ATTTTCAGCT TCCTGCTAAT CAGTGCCTCA

                                2H7 VL→
61  V I I A R G Q I V L S Q S P A I L S A S
    GTCATAATTG CCAGAGGACA AATTGTTCTC TCCAGTCTC CAGCAATCCT GTCTGCATCT

    P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

                                BamHI
                                -----
181 Y Q Q K P G S S P K P W I Y A P S N L A
    TACCAGCAGA AGCCAGGATC CTCCCCAAA CCCTGGATT ATGCCCATC CCACTGGCT

    S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTTACTC TCTCACAATC

    S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

                                (Gly4Ser)3 Linker
061 P T F G A G T K L E L K G G G G S G G G
    CCCACGTTCG GTGCTGGGAC CAAGCTGGAG CTGAAGGTG GCGGTGGCTC GGGCGGTGGT

                                2H7 VH→
421 G S G G G G S S Q A Y L Q Q S G A E L V
    GGATCTGGAG GAGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGC TGAGCTGGTG

    R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGGG CCTCAGTGAA GATGTCCTGC AAGGCTCTG GCTACACATT TACCAGTTAC

    N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAAGCA GACACCTAGA CAGGGCTTGG AATGGATTGG AGCTATTATT

    P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAATG GTGATACTTC CTACATCAG AAGTCAAGG GCAAGGCCAC ACTGACTGTA

    D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGGG

    V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTTC TGCAGAGAGT GGTGTACTAT AGTAACCTTT ACTGGTACTT CGATGCTGGG
  
```


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FIG.1B

BclI
-----human IgG1 Fc domain →

781 G T G T T V T V S D Q E P K S C D K T H
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CAGGAGCCCA AATCTTGTGA CAAAATCTCA

841 T C P P C P A P E L L G G P S V F L F P
ACATGCCAC CGTGCCAGC ACCTGAACTC CTGGGGGAGC CGTCAGTCTT CCTCTTCCCC

901 P K P K D T L M I S R T P E V T C V V V
CCAAAACCCA AGGACACCTT CATGATCTCC CGGACCCCTG AGGTACATG CGTGSTGGTG

961 D V S H E D P E V K P N W Y V D G V E V
GAGGTGAGCC ACGAAGACCC TGAGGTCAAG TTCAACTGGT ACGTGGACGG CGTGGAGGTG

1021 H N A K T K P R E E Q Y N S T Y R V V S
CATAATGCCA AGACAAAGCC GCGGGAGGAG CAGTACAACA GCACGTACCG TGTGSTCAGC

1081 V L T V L H Q D W L N G K E Y K C K V S
GTCTCTCACG TCCTGCACCA GGACTGGCTG AATGGCAAGG AGTACAAGT CAAGGTCTCC

1141 N K A L P A P I E K T I S K A K G Q P R
AACAAAGCCC TCCCAGCCCC CATCGAGAAA ACAATCTCCA AAGCCAAAGG GCAGCCCCGA

1201 E P Q V Y T L P P S R D E L T K N Q V S
GAACCAAGG TGTACACCTT GCGCCCATCC CGGGATGAGC TGACCAAGAA CCAGSTCAGC

1261 L T C L V K G F Y P S D I A V E W E S N
CTGACCTGCC TGGTCAAAGG CTCTATATCC AGCGACATCG CCGTGGAGTG GGAGAGCAAT

1321 G Q P E N N Y K T T P P V L D S D G S F
GGGAGCGCG AGAACAACTA CAGACCAAGC CCTCCCGTGC TGGACTCCGA CGGCTCTCTC

1381 F L Y S K L T V D K S R W Q Q G N V F S
TTCTCTTACA GCAAGCTCAC CGTGGACAAG AGCAGGTGGC AGCAGGGGAA CGTCTTCTCA

1441 C S V M H E A L H N H Y T Q K S L S L S
TGTCCTGTGA TGCATGAGGC TCTGCACAC CACTACAGCG AGAAGAGCCT CTCCTCTGCT

XbaI

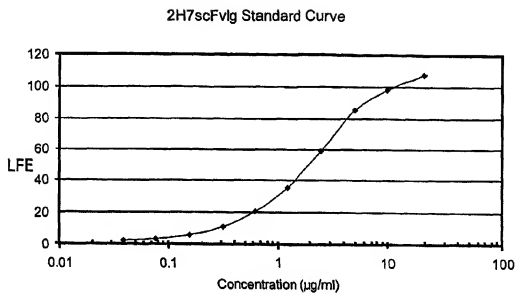
1501 P G K * S R
CCGGTAAAT GATCTAGA

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FIG.2



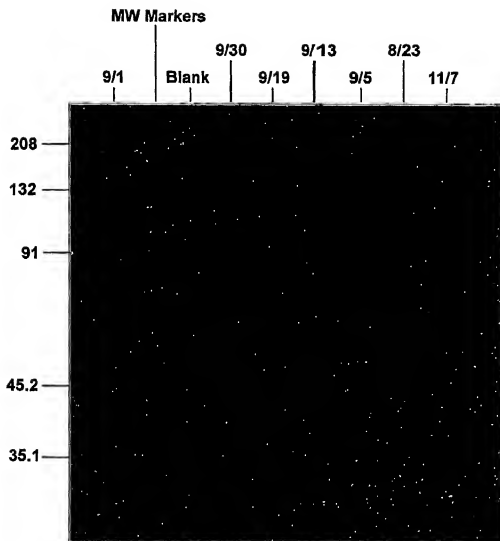
Clone	LFE @ 1:50	Estimated Concentration (µg/ml)
D2	26.1	56
IIIIC6	25.7	55
IVA3	28.6	61
Spent bulk	29.6	64

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FIG.3



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FIG. 4A

Complement Mediated B Cell Killing After Binding of CD20-targeted 2H7 Derivatives:

2H7scFv-Ig Concentration	RAMOS	BJAB
20 µg/ml + complement	0.16	0.07
5 µg/ml + complement	0.2	N.D.
1.25 µg/ml + complement	0.32	0.1
Complement alone	0.98	0.94

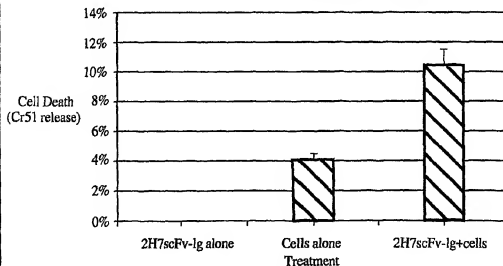
*Viability was determined by trypan blue exclusion and is tabulated as the fraction of viable cells out of the total number of cells counted.

**N.D. (not determined).

FIG. 4B

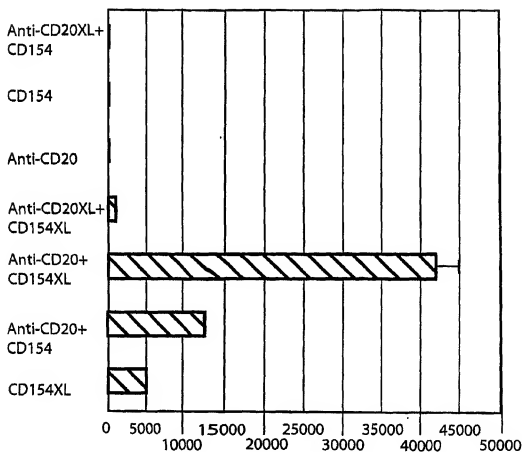
Antibody-dependent cellular cytotoxicity (ADCC) mediated by 2H7scFv-Ig:

ADCC Activity of 2H7scFv-Ig on Ramos Cells



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FIG. 5



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FIG.6A

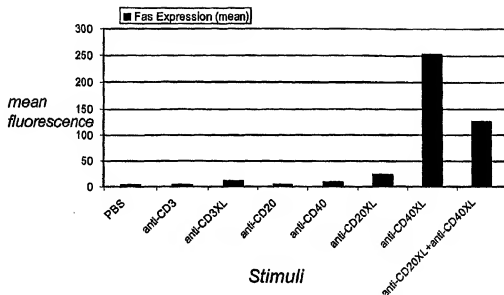
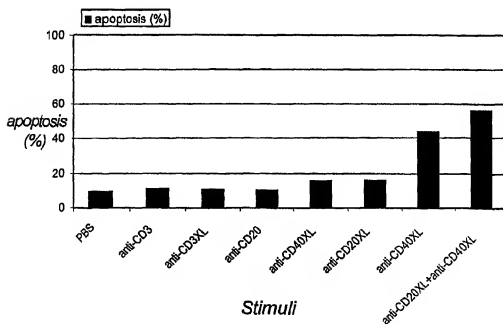


FIG.6B



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FIG. 7A

2H7-CD154 L2 cDNA and predicted amino acid sequence:

```

HindIII      NcoI  2H7 VL Leader Peptide →
-----      -
1  AAGCTTGGCCG CC  ATGGATTTC TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCTTCA

                        2H7 VL →
61  V I I A R G Q I V L S Q S P A I L S A S
    GTCATAATTC CCAGAGGACA AATGTGTTCTC TCCAGTCTC CAGCAATCCT GTCTGCATCT

    P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCACAAT GACTGCGAGG GCCAGCTCAA GTGTAAGTTA CATGCACCTGG

                        BamHI
                        -----
181 Y Q Q K P G S S P K P W I Y A P S N L A
    TACCAAGCAGA AGCCAGGATC CTCGCCCAAA CCCTGGATTTC ATGCCCCATC CAACCTGGCT

    S G V P A R F S G S G S G T S Y S L T I
241 TCTGAGTCC CTGCTCGCTT CAGTGGCAGT GGGTCTGGGA CCTCTACTC TCTACAATC

    S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTTTAACCCA

                        (Gly4Ser)3 Linker →
361 P T F G A G T K L E L K G G G G S G G G
    CCCACGTTCC GTGCTGGGAC CAAGCTGGAG CTGAAAGGTG GCGGTGGCTC GGGCGGTGGT

                        2H7 VH →
421 G S G G G G S S Q A Y L Q Q S G A E L V
    GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

    R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCATGGG CCTCAGTGAA GATGTCCTGC AAGGCTTCTG GCTACACATT TACCAGTTAC

    N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGGTAAGCA GACACCTAGA CAGGCGCTGG AATGGATTGG AGCTATTATAT

    P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

    D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGGG

    V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTCTT GTGCAAGAGT GGTGTACTAT AGTAACTCTT ACTGTACTCT CGATGTCTGG
  
```

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FIG.7B

human CD154/amino acid 48→

Bcl/Bam hybrid site

781 | G T G T T V T V S D P R R L D K I E D E
GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAAGAGGT TGGACAAGAT AGRAGATGAA

841 R N L H B D F V F M K T I Q R C N T G E
AGGAATCTTC ATGAAGATTT TGTATTCTAG AAAACGATAC AGAGATGCAA CACAGGAGAA

901 R S L S L L N C E E I K S Q F E G F V K
AGATCCTTAT CCTTACTGAA CTGTGAGGAG ATTAAAAGCC AGTTTGAAGG CTTTGTGAAG

BclI

961 D I M L N K E E T K K E N S F E M Q K G
GATATAATGT TAAACAAAGA GGAGACGAAG AAAGAAAACA GCTTTGAAAT GCAAAAAGGT

BclI

1021 D Q N P Q I A A H V I S E A S S K T T S
GATCAGATTC CTCAAATGTC GGACATGTC ATAAGTGAGG CCAGCAGTAA AACACATCT

1081 V L Q W A E K G Y Y T M S N N L V T L E
GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTTGGT ACCCTGGA

1141 N G K Q L T V K R Q G L Y Y I Y A Q V T
AATGGGAAAC AGCTGACCGT TAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

HindIII

1201 F C S N R E A S S Q A P F I A S L C L K
TTCTGTTCCA ATCGGGAAGC TTGAGTCAA GTCCCATTTA TAGCCAGCCT CTGCTAAAG

1261 S P G R F E R I L L R A A N T H S S A K
TCCCCCGGTA GATTGAGAG AATCTTACT AGAGCTGCAA ATACCCACAG TTCCGCCAA

1321 P C G Q Q S I H L G G V F E L Q P G A S
CCTTGCGGGC AACATCCAT TCACTTGGGA GGAGTATTG AATTGCAACC AGGTGCTTG

NcoI

1381 V F V N V T D P S Q V S H G T G F T S F
GTGTTTGCA ATGTACTGA TCCAAGCCAA GTGAGCCATG GCATGGCTT CACGTCTTT

XhoI XbaI

1441 G L L K L E * * S R
GGCTTACTCA AACTCGAGTG ATAATCTAGA

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FIG. 7C

2H7scFv-CD154 S4 cDNA and predicted amino acid sequence:

```

HindIII      NcoI
-----      -
-----2H7 VL Leader Peptide→
      M D P Q V Q I F S F L L I S A S
1 AAGCTTGCCG CC ATGGATT TCAAGTGCAG ATTTTCAGCT TCCTGCTAAT CAGTGCCTCA

      2H7 VL →
      V I I A R G Q I V L S Q S P A I L S A S
61 GTCATAATTG CCAGAGGACA AATGTTCTC TCCCAGTCTC CAGCAATCCT GTCTGCATCT

      P G E K V T M T C R A S S S V S Y M H W
121 CCAGGGGAGA AGGTCACAAT GACTTGCAGG GCCAGCTCAA GTGTAAGTTA CATGCACTGG

      BamHI
-----
      Y Q Q K P G S S P K P W I Y A P S N L A
181 TACCAGCAGA AGCCAGGATC CTCCCCCAA CCTGGATT TATGCCCATC CAACCTGGGT

      S G V P A R F S G S G S G T S Y S L T I
241 TCTGGAGTCC CTGCTGCTT CAGTGGCAGT GGTCTGGGA CCTCTTACTC TCTCACAATC

      S R V E A E D A A T Y Y C Q Q W S F N P
301 AGCAGAGTGG AGGCTGAAGA TGCTGCCACT TATTACTGCC AGCAGTGGAG TTITAACCCA

      (Gly4Ser)3 Linker →
      P T F G A G T K L E L K G G G G S G G G
361 CCCACGTTG GTGCTGGAC CAAGCTGGAG TGAAAGGTG GCGGTGGCTC GGGCGGTGGT

      2H7 VH →
      G S G G G G S S Q A Y L Q Q S G A E L V
421 GGATCTGGAG GAGGTGGGAG CTCTCAGGCT TATCTACAGC AGTCTGGGGC TGAGCTGGTG

      R P G A S V K M S C K A S G Y T F T S Y
481 AGGCCTGGG CCTCAGTGA GATGCTCTG AAGGCTTCTG GCTACACATT TACCAGTTAC

      N M H W V K Q T P R Q G L E W I G A I Y
541 AATATGCACT GGTAAAGCA GACACCTAGA CAGGCTCTGG AATGGATTGG AGCTATTATT

      P G N G D T S Y N Q K F K G K A T L T V
601 CCAGGAATG GTGATACTTC CTACAATCAG AAGTTCAAGG GCAAGGCCAC ACTGACTGTA

      D K S S S T A Y M Q L S S L T S E D S A
661 GACAAATCCT CCAGCACAGC CTACATGCAG CTCAGCAGCC TGACATCTGA AGACTCTGGG

      V Y F C A R V V Y Y S N S Y W Y F D V W
721 GTCTATTICT GTGCAAGAGT GGTGTACTAT AGTAACCTTT ACTGGTACTT CGATGTCTGG
  
```

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FIG. 7D

human CD154/amino acid 108 →

```

                                Bcl/Bam hybrid site          BclI
781  G T G T T V T V S D P E N S F E M Q K G
    GGCACAGGGA CCACGGTCAC CGTCTCTGAT CCAGAAAACA GCTTTGAAAT GCAAAAAGGT

    BclI
    ~~~~~
841  D Q N P Q I A A H V I S E A S S K T T S
    GATCAGAATC CTCAAATTGC GGCACATGTC ATAAGTGAGG CCAGCAGTAA AACACATCT

    V L Q W A E K G Y Y T M S N N L V T L R
901  GTGTTACAGT GGGCTGAAAA AGGATACTAC ACCATGAGCA ACAACTGGT AACCCGGAA

    N G K Q L T V K R Q G L Y Y I Y A Q V T
961  AATGGGAAC AGCTGACCGT TAAAAGACAA GGACTCTATT ATATCTATGC CCAAGTCACC

                                HindIII
                                ~~~~~
1021 F C S N R E A S S Q A P F I A S L C L K
    TTCTGTTCCA ATCGGGAAGC TTCGAGTCRA GCTCCATTTA TAGCCAGCCT CTGCTTAAG

    S P G R F E R I L L R A A N T H S S A K
1081 TCCCCCGTA GATTCGAGAG AATCTTACTC AGAGCTGCAA ATACCCACAG TTCGCCAAA

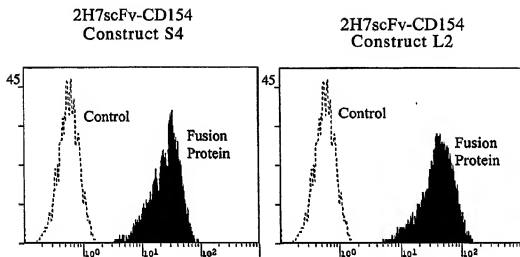
    P C G Q Q S I H L G G V F E L Q P G A S
1141 CCTTGGGGC AACATCCAT TCACCTGGGA GGAGTATTG AATTGCAACC AGGTGCTTCG

                                NcoI
                                ~~~~~
1201 V F V N V T D P S Q V S H G T G F T S F
    GTGTTTGTA ATGTGACTGA TCAGAGCCAA GTGAGCCATG GCACTGGCTT CACGTCCTTT

                                XhoI          XbaI
                                ~~~~~
1261 G L L K L E * * S R
    GGCTTACTCA AACTCGAGTG ATAATCTAGA
  
```

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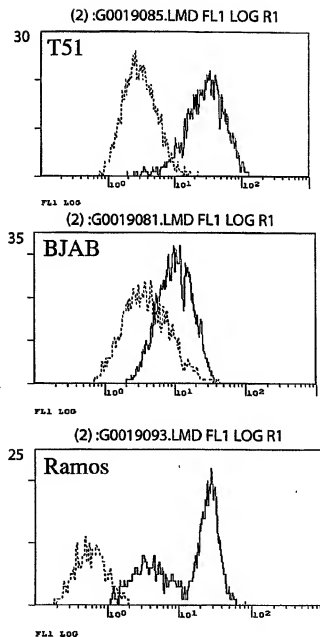
FIG.8



CD20 CHO cell targets + (control or fusion protein)
+ Biotin-CD40Ig + PE-SA

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FIG.9



.....control supernatant 2H7scFv-CD154 supernatant

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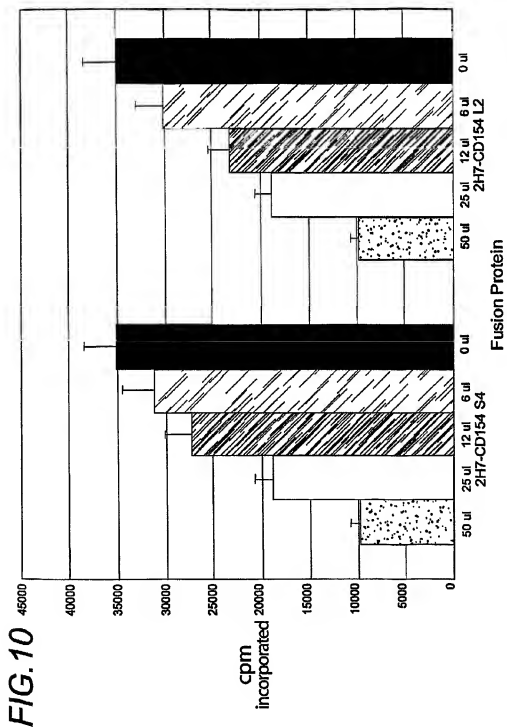
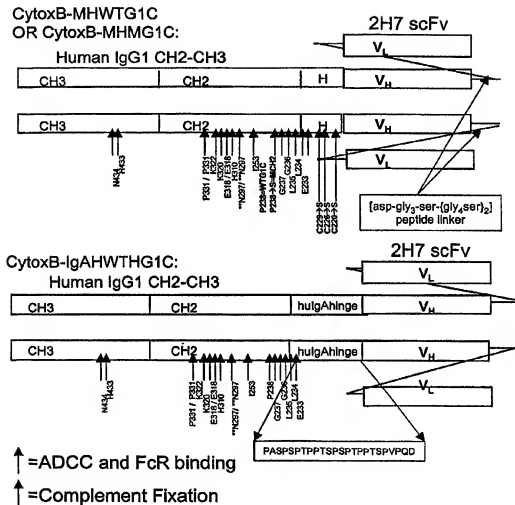
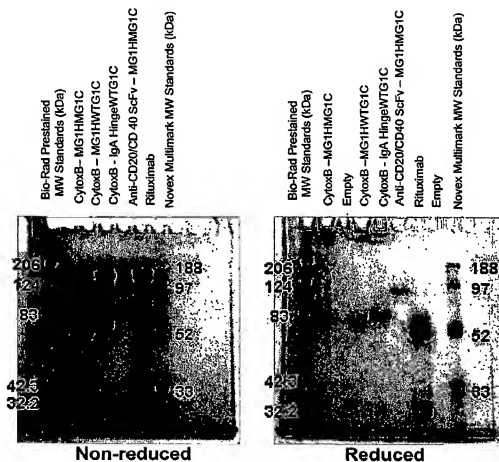


FIG. 11



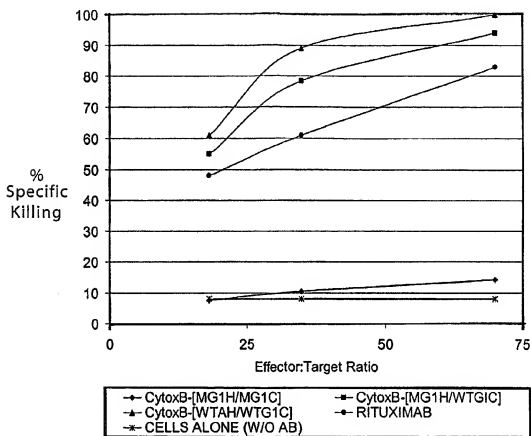
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FIG. 12



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FIG. 13

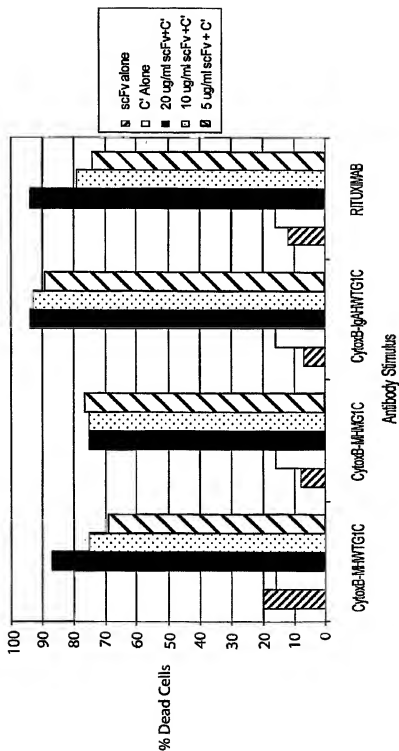


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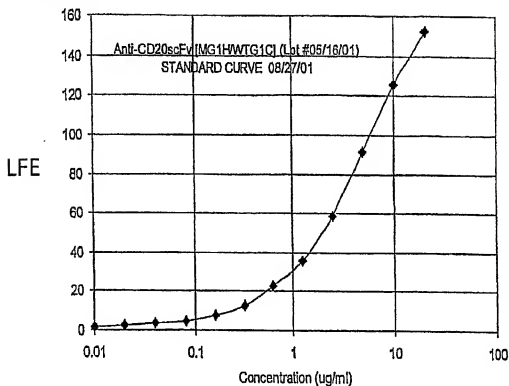
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FIG. 14



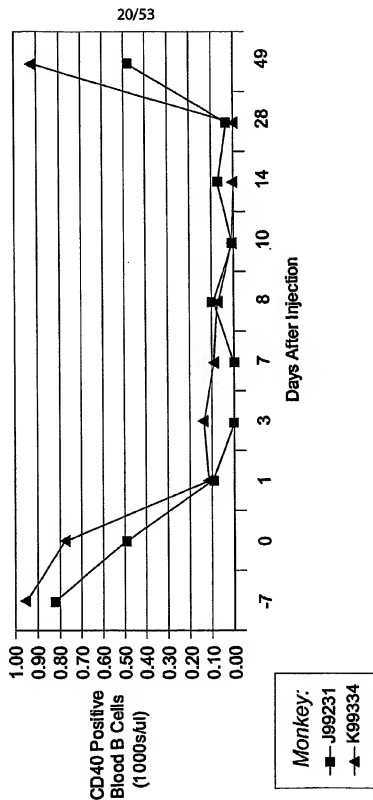
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FIG. 15



	Day	Monkey J99231		Monkey K99334	
		LFE(1:40)	Concentration (μ g/mL)	LFE(1:40)	Concentration (μ g/mL)
Injection \rightarrow	-7	2.41	<0.6 μ g/mL	1.51	<0.4 μ g/mL
	0	2.22	<0.6 μ g/mL	1.63	<0.4 μ g/mL
	1	73.8	220 μ g/mL	44.4	100 μ g/mL
Injection \rightarrow	3	20.0	28 μ g/mL	40.2	80 μ g/mL
	7	15.6	24 μ g/mL	15.7	24 μ g/mL
	8	39.1	80 μ g/mL	42.6	92 μ g/mL
	10	11.5	18 μ g/mL	2.74	1.2 μ g/mL
	14	2.05	0.6mg/mL	1.96	0.6 μ g/mL

FIG. 16

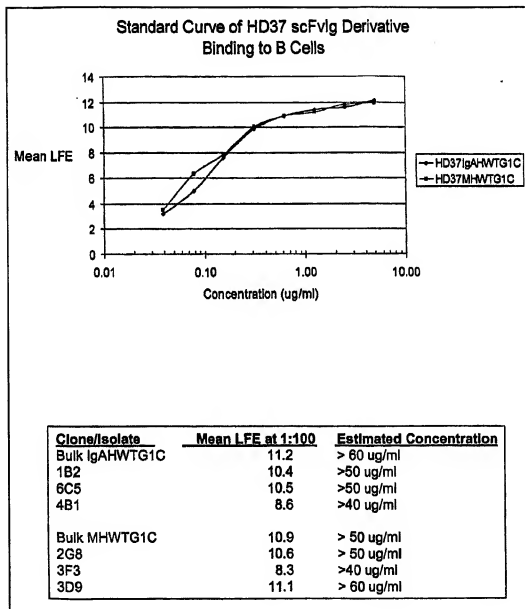


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FIG. 17



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FIG.18

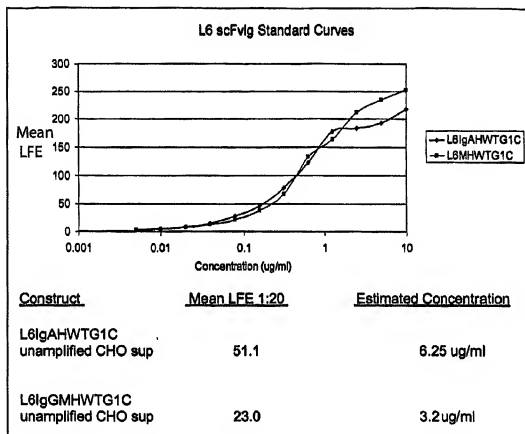


FIG. 19A

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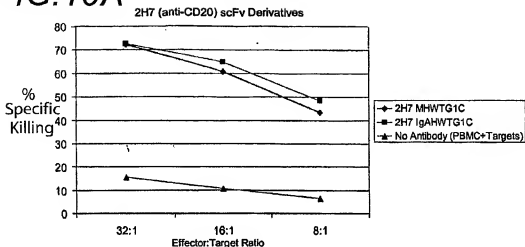


FIG. 19B

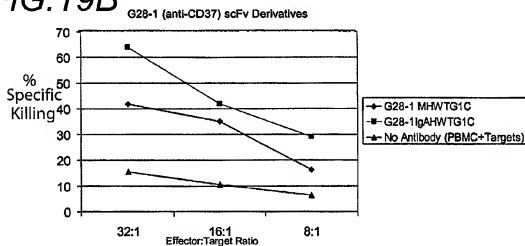
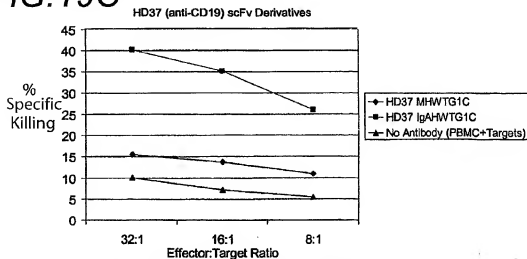
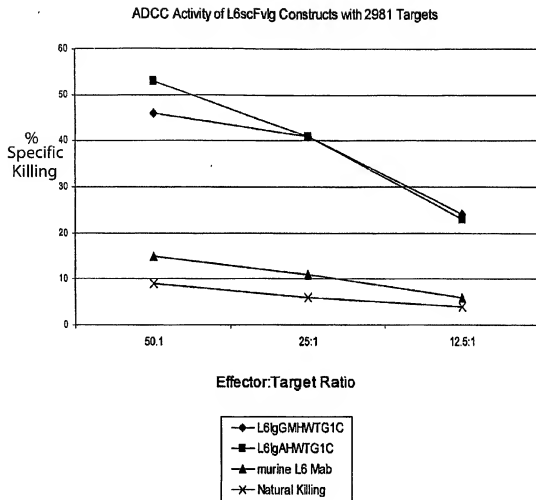


FIG. 19C



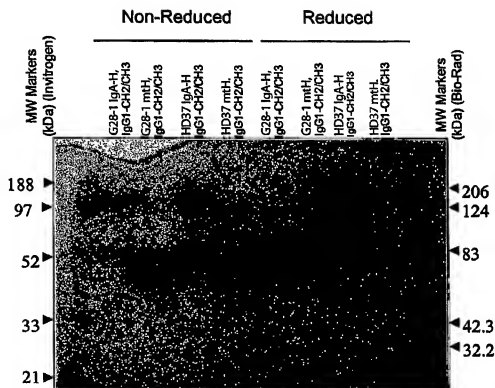
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FIG.20



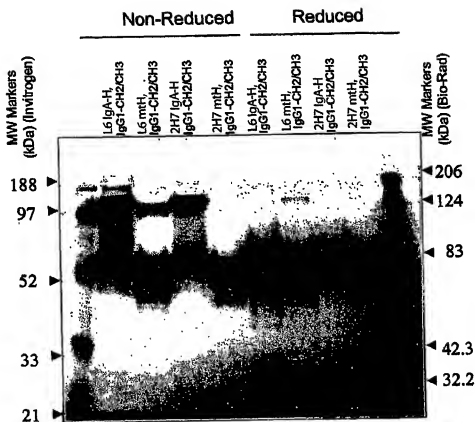
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FIG.21



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FIG.22



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FIG.23

Hinge

CH2

Human IgG1: -----HTCPPC PAPELLSGPSVFLPPPKPDITMISRTPEVTCVVVDVSHEDPEVKFNWYDG
Llama IgG2: DQEPKTPRPQPPQPNPTTESKCFKC PAPELLSGPSVFLPPPKPDVLSISGRPEVTCVVVDVQGEDPEVSNWYIDG
Llama IgG1: --EPHGG-----CTCPQC PAPELLSGPSVFLPPPKPDVLSISGRPEVTCVVVDVQGEDPEVSNWYIDG
Llama IgG3: --AHHSEDT-----SKCFKC PGPELLSGPTVFIFPPPKADWLISITRKPEVTCIMWTWVKKTIIRSSSSNSVDD

CH3

VEVHNAKTFRREEQNSTYRVVSVLPIQHQDWLNGKEYCKCKVNNKALPAPIERTISKAGQTRFPQVYITLAPSRDELTRKQVSLT
TAEVTRANTRPKEEQFNSTYRVVSVLPIQHQDWLNGKEYCKCKVNNKALPAPIERTISKAGQTRFPQVYITLAPHREELAKDTVSVT
VEVTRANTRPKEEQFNSTYRVVSVLPIQHQDWLNGKEYCKCKVNNKALPAPIERTISKAGQTRFPQVYITLAPHREELAKDTVSVT
TEVHTAETKRPKEEQFNSTYRVVSVLPIQHQDWLNGKEYCKCKVNNKALPAPIERTISKAGQTRFPQVYITLAPHREELAKDTVSVT

CLVKGFTPSDIAVEWESNGQPEN--NYKTTTPPVLDSDGSFFLYSKLTVDKSNWQGNVFSVSMHEALHNHYTOKSLSPGK
CLVKGFTPPDINVEWQRNGQPSSEGTANTPPQLINDGTFFLXSKXSVGKNTWQQGETTCVVMHEALHNHYTOKSLTQSSGK
CLVKGFTPADINVEWQRNGQPSSEGTANTPPQLINDGTFFLYSKLXSVGKNTWQQGETTCVVMHEALHNHYTOKSLTQSSGK
CLVKGFTPADINVEWQRNGQPSSEGTANTPPQLINDGTFFLYSKLXSVGKNTWQQGEVFTCVVMHEALHNHYTOKSLTQSSGK

FIG.24

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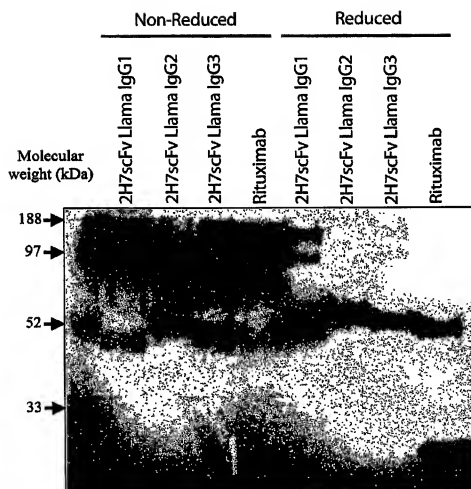
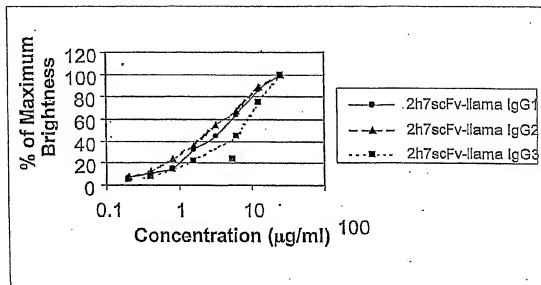
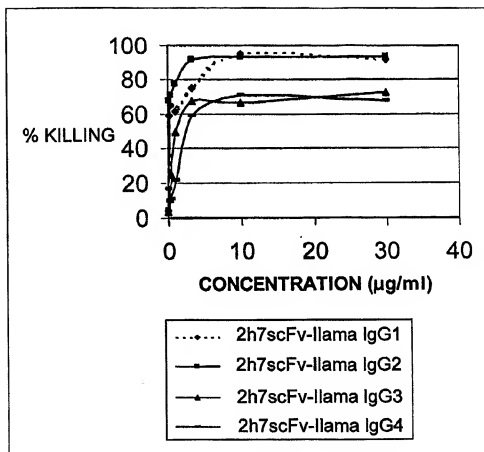


Figure 25



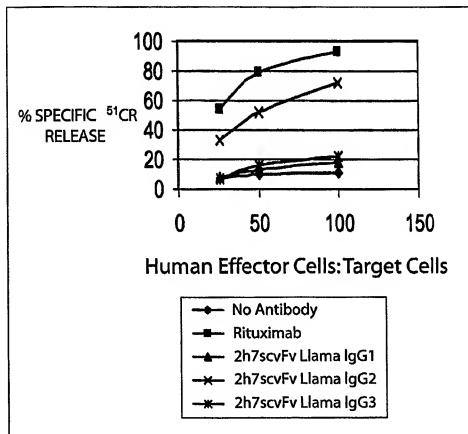
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FIG. 26



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FIG.27



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FIG.28

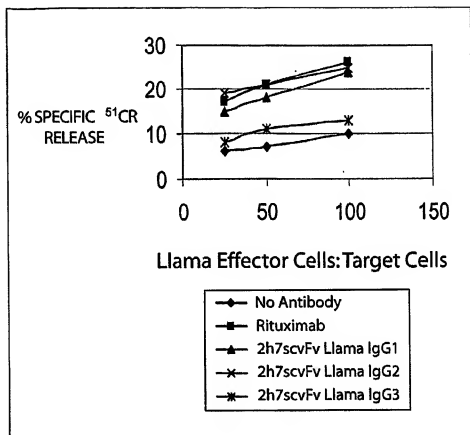
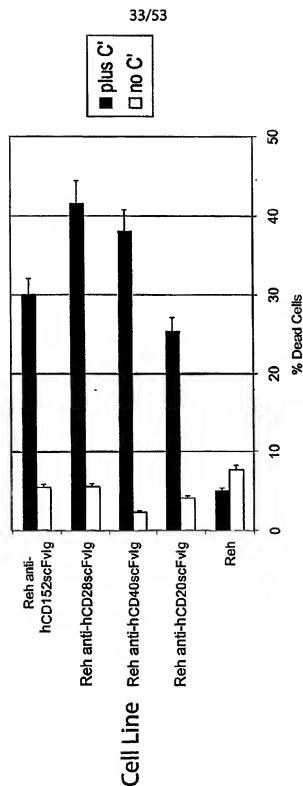
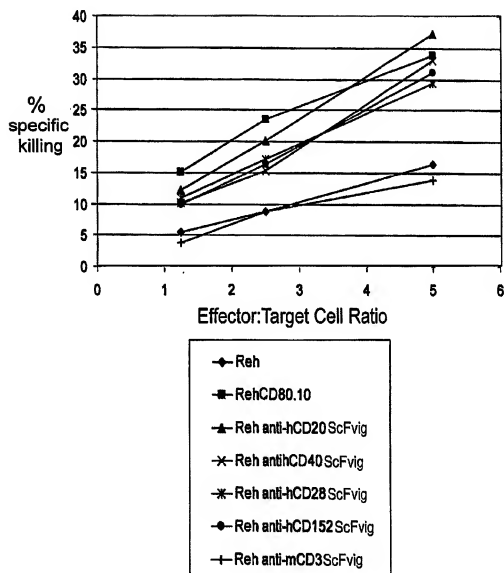


FIG. 29



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FIG. 30

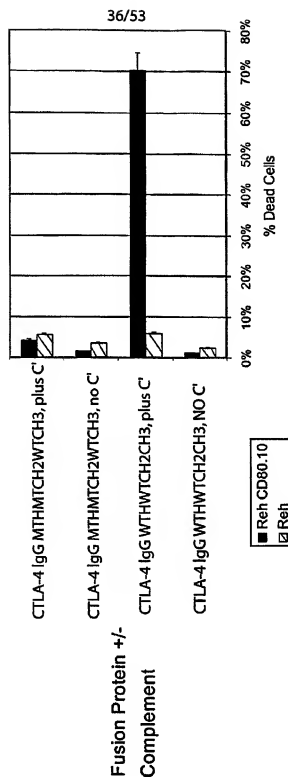


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FIG.31

Name Identifier	Hinge Sequence	CH2 Sequence	CH3 Sequence	SEQ ID NO:
IgG WTH (CCC) WTH2CH3	IgG1 WT Hinge (CCC)	Wild Type CH2	Wild Type CH3	
IgG MTH (SSS) WTH2CH3	IgG1 Mutant Hinge (SSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
VH SER 11 IgG MTH (SSS) WTH2CH3	IgG1 Mutant Hinge (SSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgG (SSC) WTH2CH3	IgG1 Mutant Hinge (SSC)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgG (SCS) WTH2CH3	IgG1 Mutant Hinge (SCS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgG (CSS) WTH2CH3	IgG1 Mutant Hinge (CSS)	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgG MTH(SSS) MTH2WTH3	IgG1 Mutant Hinge (SSS)	Mutant CH2 (IgG1) Pro ? Ser 238	Wild type CH3 (IgG1)	
IgAH IgGWTCH2CH3	IgA Hinge	Wild type CH2 (IgG1)	Wild type CH3 (IgG1)	
IgAH IgACH2CH3	IgA Hinge	Wild type CH2 (IgA)	Wild type CH3 (IgA)	
IgAH IgA-T 4	IgA Hinge	Wild type CH2 (IgA)	Truncated CH3 (IgA) (deletion of 4 amino acids at carboxy terminus)	

FIG.32



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FIG.33A

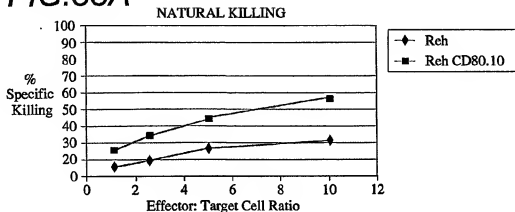


FIG.33B

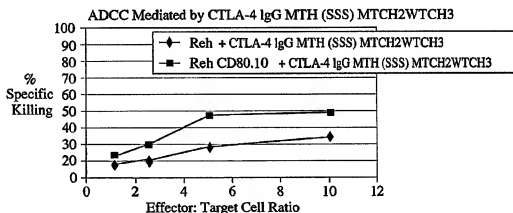
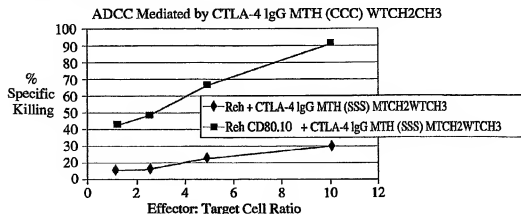


FIG.33C

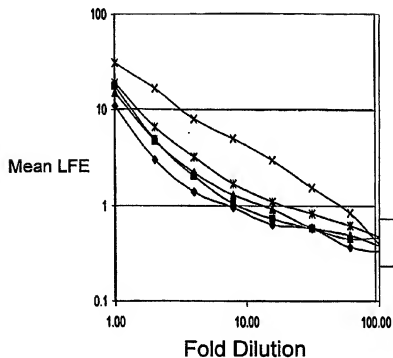


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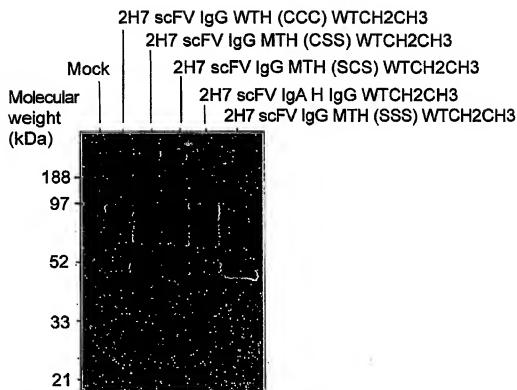
FIG.34



- ◆ 2H7 scFv WTH (CCC) WTCH2CH3
- 2H7 scFv MTH (CSS) WTCH2CH3
- ▲ 2H7 scFv MTH (SCS) WTCH2CH3
- * 2H7 scFv MTH (SSC) WTCH2CH3
- ✱ 2H7 scFv VH11SER WTH (CCC) WTCH2CH3

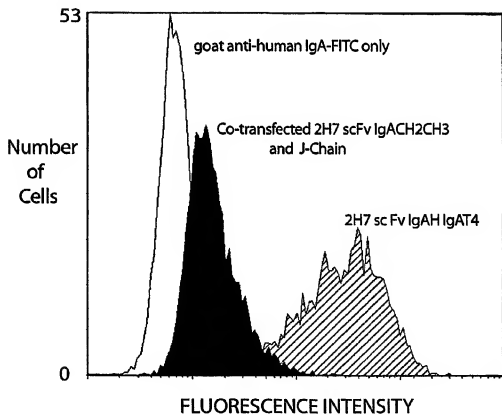
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FIG.35



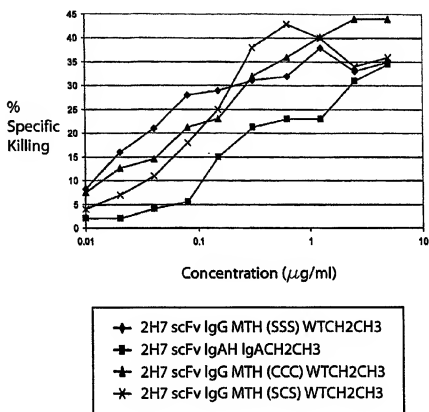
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FIG.36



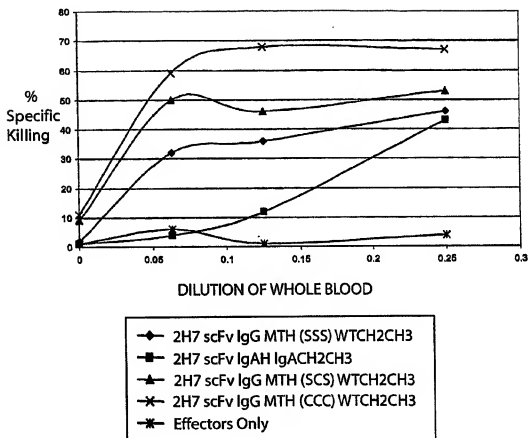
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FIG.37



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FIG.38



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FIG.39A

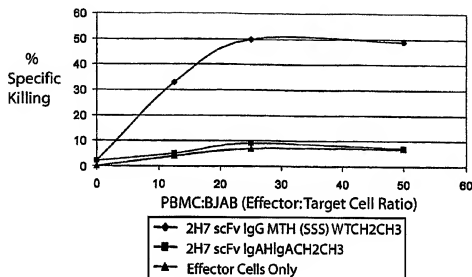
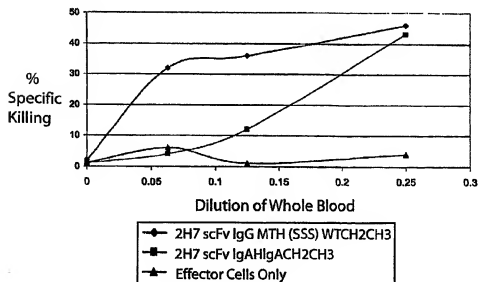


FIG.39B



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FIG. 40

